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**13. ABSTRACT** ***(Maximum 200 Words)***

This report summarizes research on corneal damage from exposures to multiple pulses of $CO_2$ laser radiation at 10.6 μm and Tm:YAG laser radiation at 2.02 μm. Retinal damage from sources with rectangular irradiance distributions was also modeled. Thresholds for $CO_2$ exposures were determined for sequences of up to 1024 pulses (80 ns duration) at frequencies of 10 and 16 Hz. Thresholds for Tm:YAG exposures were determined sequences of up to 128 pulses (0.300 s duration) at 1 Hz, and for sequences of up to 999 pulses at 10 and 20 Hz (0.025 s duration ) and 100 Hz (0.005 s duration). Thresholds are correlated by an empirical power law relationship between either the energy density per pulse ($CO_2$) or the irradiance (Tm:YAG) and the number of pulses. For some Tm:YAG exposures the law underestimates the damage threshold for small numbers of pulses. Thresholds for single pulses of Tm:YAG radiation and multiple pulses of $CO_2$ radiation also are described by a critical temperature model. A critical temperature model sometimes, but not always, describes threshold damage from sequences of pulses of Tm:YAG radiation. Predicted retinal damage thresholds for rectangular irradiance distributions were compared to those for Gaussian beams having equal 1/e areas. Agreement was excellent for beams with low aspect ratios; but only fair for beams with high aspect ratios where the effects of lateral heat conduction become more important at longer exposures.

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Russell R. McCulley 4/5/2000

P.I. Signature Date

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# Introduction

The contract with USAMRMC was signed November 9, 1995. After establishing budgets we conferred with the Contracting Officer's Representative, Mr. Bruce Stuck, to reaffirm his research needs and priorities. In this regard it should be noted that the Statement of Work for the first year, dated February 24, 1995, was the same as in the original proposal for this research that we had submitted in 1991. We wanted to confirm that the research described there was still appropriate. The Statement of Work was:

1. The data base of threshold conditions for 80 ns pulses from $CO_2$-TEA lasers will be extended by measuring the threshold energy densities for sequences of 32, 128 and 1024 pulses at 10 Hz and sequences of 2, 8, 32 and 128 pulses at 20 Hz. The existing thresholds for 2 and 8 pulses at 10 Hz will be refined.
2. An understanding of the damage mechanism for such pulses will be pursued by:

   a) Determining how lowering the temperature of the epithelium affects the threshold,

   b) Obtaining light and electron micrographs of the damage,

   c) Using high-speed photography to investigate ablation of material from the corneal surface, and

   d) Measuring pressure transients in model systems.
3. The required characteristics of potential near-infrared laser source(s) for damage studies will be identified by performing detailed temperature calculations in the 1.3 to 2.5 μm wavelength region.

Mr. Stuck confirmed that the multiple-pulse damage threshold determinations noted there were still of major interest as were investigations of potential corneal damage in the 1.3 to 2.5 μm spectral region. He suggested that we place less emphasis on damage mechanisms.

In 1997, the Simulation Training and Instrumentation Command (STRICOM) and the U.S. Army Center for Health Promotion and Preventative Medicine (CHPPM) identified a soldier health and safety research issue with regard to the fielding of the MILES 2000 systems. They stated that additional biological effects modeling was required to assess the functional dependence ocular injury thresholds for exposures from sources that produce rectangular irradiance distributions such as the laser diodes used in the MILES 2000 systems. A contract modification was made on Jan. 30, 1998 to address this issue.

# Body

## $CO_2$-TEA Laser Damage Thresholds

The detailed experimental methods for characterizing the laser and determining damage thresholds are described in Appendices A and B. in accordance with the Statement of Work we repeated the determination of the damage thresholds for 2 and 8 pulses at 10 Hz. The refined threshold for two pulses (c.f., Table 1) is slightly higher than the value of 200 mJ/cm$^2$/pulse that we had determined in preliminary experiments performed under a previous contract from the

Army Medical Research and Development Command.[1, 2] We found that the threshold for 8 pulses at 10 Hz (c.f., Table 1) was the same as we previously reported.[1, 2] Table 1 lists damage thresholds for sequences of 2, 8, 32, 128 and 1024 pulses at pulse repetition frequencies of 10 and 16 Hz.*

**Table 1:** Threshold energy densities and calculated maximum temperature rises for sequences of 80 ns pulses.

| Number of Pulses | Pulse Repetition Frequency (Hz) | $ED_{th}$ (mJ-cm$^{-2}$-pulse$^{-1}$) | $d_{1/e}$ (mm) | $\Delta T_{max}$ (C)[a] |
|---|---|---|---|---|
| 1 | – | 307 | 3.72 | 30.25 |
| 2 | 10 | 235 | 3.48 | 25.68 |
| 8 | 10 | 228 | 3.80 | 32.00 |
| 32 | 10 | 154 | 3.78 | 29.15 |
| 128 | 10 | 136 | 3.41 | 32.45 |
| 1024 | 10 | 95 | 3.21 | 26.60 |
| 2 | 16 | 265 | 3.62 | 29.73 |
| 8 | 16 | 205 | 3.75 | 31.26 |
| 32 | 16 | 150 | 3.73 | 32.90 |
| 128 | 16 | 105 | 3.82 | 32.80 |
| 1024 | 16 | 85 | 3.58 | 35.06 |

[a] Calculated on the beam axis, 10 μm beneath the anterior tear surface.

In order to perform the experiments with 32 or more pulses it was necessary to put a partially reflecting attenuator in the laser beam because the required pulse energies were below the lasing threshold. We found that the beam diameter measured with the partial reflector in

* In the original Statement of Work we were to determine epithelial damage thresholds at a pulse repetition frequency of 20 Hz. However, we discovered that the output of the Boston Laser (Model 220S) was unstable at pulse frequencies above 16 Hz (although the specifications claimed 20 Hz). Boston Laser is no longer in business, consequently technical support for the laser was not available. We discussed this problem with our Contracting Officer's Representative, Mr. Bruce Stuck, and it was agreed that we would make the determinations at 16 Hz (note that we also made an additional threshold determination for 1024 pulses at 16 Hz that was not included in the original Statement of Work).

place was smaller than when it was removed. This led us to question the single-pulse threshold we previously reported,[1, 2] because at the time it was determined our practice was to attenuate the beam so that the detector used to measure beam diameter would not be saturated. We noted that if the unattenuated beam used in determining the single-pulse threshold actually had a larger diameter than we measured with the attenuator in place, then the correct value of $ED_{th}$ would in fact be lower. Consequently we repeated the single-pulse threshold measurement without using the attenuator. In these new experiments, the beam intensity was reduced by decreasing the laser power. We found that the threshold was indeed lower - 307 mJ-cm$^{-2}$ compared to 360 mJ-cm$^{-2}$ which was found previously.[1, 2]

Figure 1 shows a laser lesion caused by an exposure to 128 pulses at 16 Hz. The energy density for this exposure was 144 mJ-cm$^{-2}$, which is 30 percent greater than the damage threshold. Damage is confined to the epithelium.

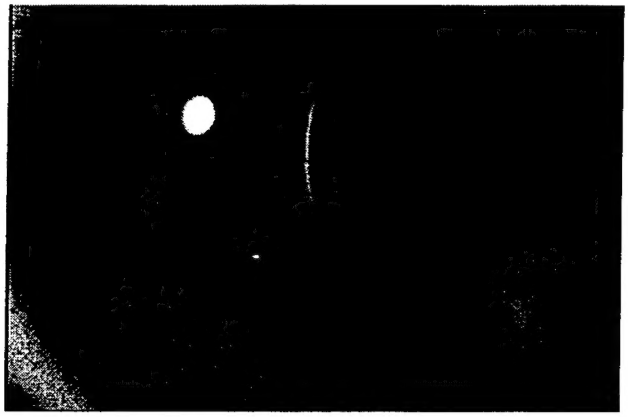

**Figure 1:** Lesion resulting from an exposure to 128 pulses from a $CO_2$-TEA laser at a pulse repetition frequency of 16 Hz. The energy density was 144 mJ-cm$^{-2}$, which is 30 percent greater than the damage threshold.

The damage threshold energy densities per pulse listed in Table 1 are plotted in Figure 2 as a function of the number of pulses. Least squares fits to these data show that the thresholds at 10 Hz and 16 Hz are both correlated by an empirical power law of the form

$$ED_{th} = CN^{-\alpha}, \qquad (1)$$

where $N$ is the number of pulses in the sequence. The empirical constants $C$ and $\alpha$ are determined by a least-squares fit to the experimental data. For the 10 Hz thresholds, $C$ = 291 mJ-cm$^{-2}$-pulse$^{-1}$ and $\alpha$ = 0.162 (R = 0.976),[3, 4] and for the 16 Hz thresholds, $C$ = 300 mJ-cm$^{-2}$-pulse$^{-1}$ and $\alpha$ = 0.194 (R = 0.997). It is not possible to discern if the slight difference between the two fits is real; but, in any case, both fits fall within the ±10 percent accuracy estimated from the bracketing procedure used to determine the thresholds (see Appendix A). If both sets of data are

assumed to be part of the same population, the fitting procedure shows that $C = 295.5$ mJ-cm$^{-2}$-pulse$^{-1}$ and $\alpha = 0.178$ (R = 0.984). The values for the constant $C$ all differ by less than 5 percent from the measured damage threshold energy density for a single pulse. This is well within the estimated accuracy of the procedure used for determining individual thresholds.

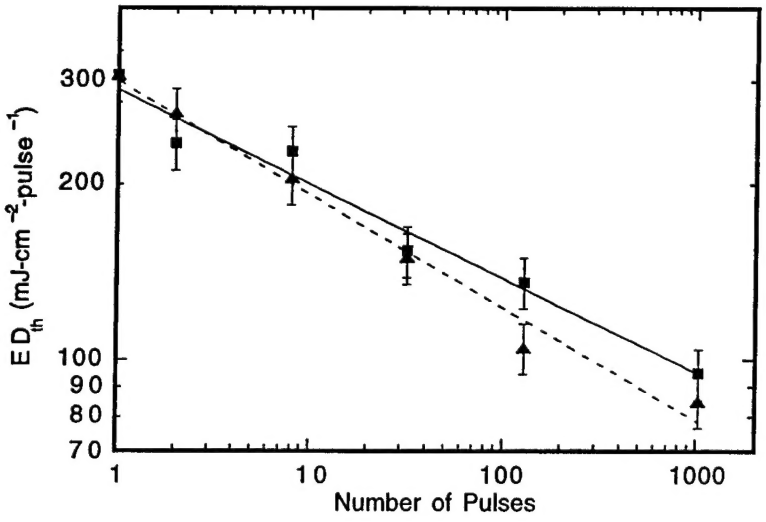


**Figure 2:** The dependence of the threshold energy density per pulse on the number of pulses at pulse frequencies of 10 and 16 Hz. The lines are least-squares fits to a power law of the form $ED_{th} = CN^{-\alpha}$. The corresponding values of $C$ and $\alpha$ are given in the text. The error bars are ±10 percent of the experimental threshold values (■ – 10 Hz, ▲ – 16 Hz) and represent the estimated accuracy of the bracketing procedure used to determine the thresholds.

The power law is of the same form that we found previously for sequences of pulses having individual pulse durations between 0.001 and 1 s with pulse repetition frequencies between 1 and 100 Hz. However, for the longer pulse durations, the exponent $\alpha$ was ~ 0.25 and the coefficient depended on the duration of the individual pulses.[5] Coincidentally, retinal damage thresholds for sequences of pulses are also described by a power law of this same form with $\alpha = 0.25$.[6]

The maximum temperature increases calculated for the damage threshold exposures are also listed in Table 1. Temperatures are calculated at a position on the beam axis, 10 μm beneath the surface of the tear layer. Thus, assuming that the tear layer is about 7 μm thick,[7] the temperature increases are those that occur just inside the anterior-most epithelial cells. Because of heat conduction, the temperature at this position does not reach its maximum value until about

164 μs after the final pulse. Figure 3 shows two illustrative temperature histories calculated for damage threshold exposures for sequences of 32 and 128 pulses at a pulse repetition frequency of 16 Hz.

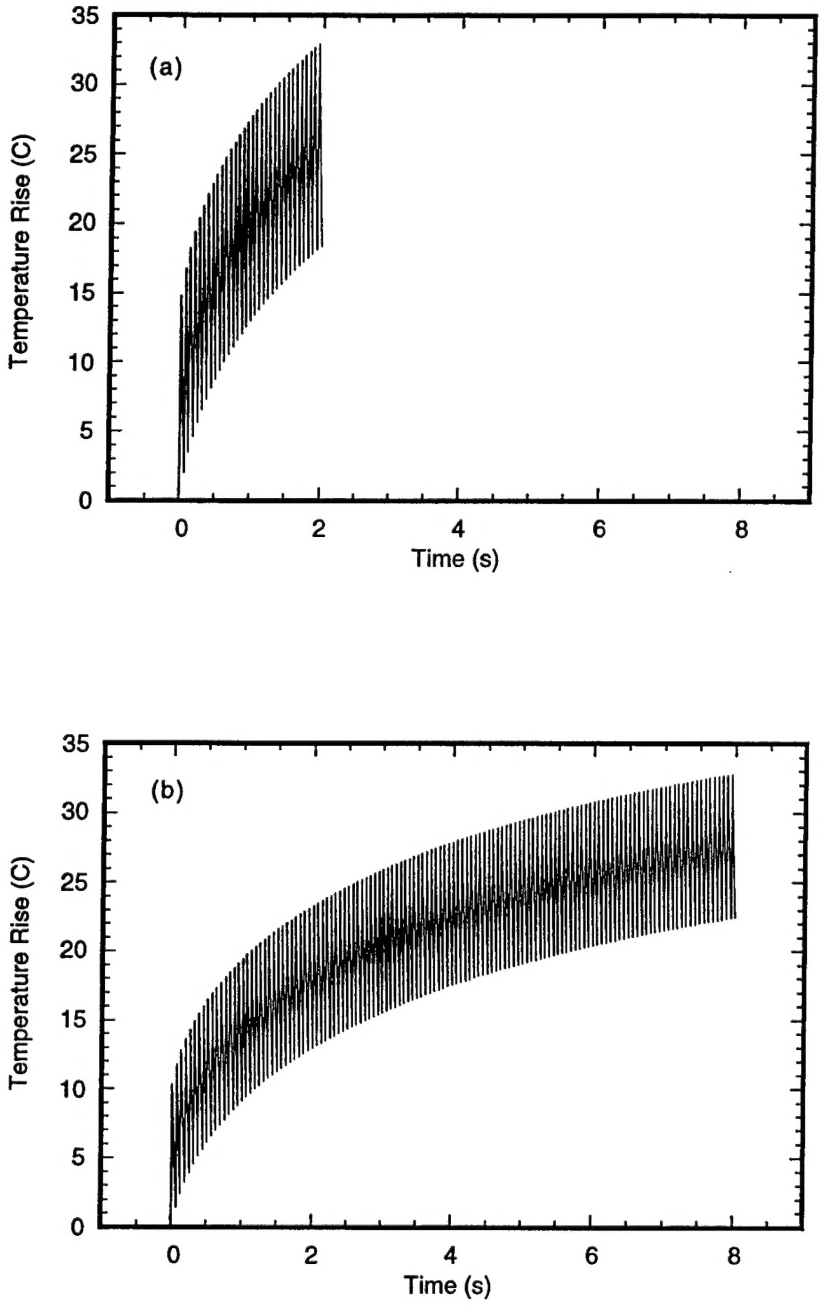


**Figure 3:** Calculated temperature histories on the beam axis 10 μm beneath the tear surface at the damage threshold exposure for sequences of 32 pulses, and (b) 128 pulses at 16 Hz.

The maximum temperature increases calculated for both exposure conditions (viz., 10 and 16 Hz) are constant to within ± 10 percent of their mean values, independent of the number of pulses. For the exposures at 10 Hz, $\Delta T_{max}$ = 29.4±2.8 C (mean±SD), and for the exposures at 16 Hz, $\Delta T_{max}$ = 32.0±2.0 C. The maximum temperature increases for the two exposure conditions are shown graphically in Figure 4. The fact that the calculated maximum temperature increases are essentially constant for all of damage thresholds at a given frequency suggests that the damage mechanism has a substantial thermal component and can be described by a critical temperature damage model.

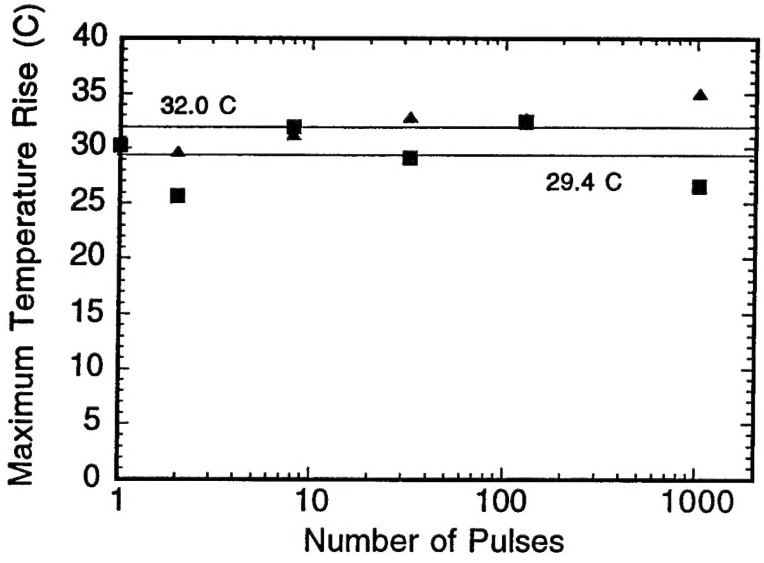


**Figure 4:** The calculated maximum temperature rises on the beam axis, 10 μm beneath the tear surface for the damage threshold exposure. The lines show the mean values of $\Delta T_{max}$ for the two exposure conditions (■ – 10 Hz, ▲ – 16 Hz).

**$CO_2$-TEA laser damage mechanism.**

In order to elucidate the damage mechanism further, a series of damage experiments was performed on enucleated eyes that were cooled to room temperature (average 21 C) as described in the Materials and Methods section of Appendix B. The underlying hypothesis for this test is that if a critical temperature model is valid, then damage should occur for exposures that result in the same final critical temperature (not temperature increase). Thus for a cornea initially at room temperature sufficient additional energy would have to be supplied, first to raise the temperature to the *in vivo* temperature, and then to the final "damage" temperature. In a preliminary experiment using 8 pulses at 16 Hz, corneas in cooled enucleated eyes did not incur damage at the same (slightly above threshold) exposure that produced lesions in corneas *in vivo*. This finding suggested that the damage mechanism was at least partially thermal. Subsequently, damage thresholds for cooled corneas were determined for exposures to sequences of 8 and 32

pulses at 16 Hz. The threshold energy densities and the calculated maximum temperature rises for these experiments are listed in Table 2. If one assumes that the temperature of the anterior surface of a cornea *in vivo* is 35 C,[8] the "damage temperatures" from Table 1 for the 8 and 32 pulse 16 Hz thresholds would be respectively 339.5 K and 341.1 K; whereas they are respectively 354 K and 345.8 K for the corneas that were cooled to 21 C before exposure. The additional energy required to produce a minimal lesion in the cooled corneas is therefore sufficient to raise the cornea temperature to a level slightly higher than that associated with damage *in vivo*. These results suggest that threshold damage from exposures to multiple pulses does have a substantial thermal component. The higher "damage temperatures" in the experiments on cooled enucleated eyes are possibly due a slowing down of the processes leading to the observed damage endpoint as a result of the lower ambient temperature. (Recall that the damage assessment is made 1/2 hour after exposure and the enucleated eyes are maintained in BSS at 21 C during this time, whereas the *in vivo* eyes are at their normal, or perhaps slightly higher temperature during this time, because they are taped shut.)

**Table 2:** Threshold Energy Densities and Calculated Maximum Temperature Rises for Enucleated Eyes at 21 C.

| Number of Pulses | Pulse Repetition Frequency (Hz) | $ED_{th}$ (mJ-cm$^{-2}$-pulse$^{-1}$) | $d_{1/e}$ (mm) | $\Delta T_{max}$ (C) [a] |
|---|---|---|---|---|
| 8 | 16 | 393 | 3.58 | 59.8 |
| 32 | 16 | 236 | 3.58 | 51.6 |

[a] Calculated on the beam axis, 10 μm beneath the anterior tear surface.

Nevertheless, in preliminary experiments performed under a previous contract from the Army Medical Research and Development Command we noted that light and electron micrographs of corneas exposed to a single 80 ns pulse just above the damage threshold had features consistent with both thermal and mechanical (e.g., acoustic) damage.[1, 2] These, as well as the present experiments were conducted under conditions where the energies were too low to cause optical breakdown or tissue ablation; however, large temperature gradients at the anterior surface resulting from these short exposures could produce thermoelastic pressure transients.[9-12] The thermoelastic stress wave generated by laser absorption at a free surface is bipolar; i.e., it consists of a compression wave followed by a tensile wave.[10-12] According to A. G. Doukas (private communication), tensile stress is more damaging than compressional stress. According to him, rise times and other conditions being equal, it takes about an order of magnitude less tensile stress amplitude to cause cellular damage than it does for compressional stress; however, there are no published data to support this contention. Disruptions of the superficial wing cells of the epithelium noted by Farrell et al[1, 2] are consistent with the type of effect that might result

from the passage of a tensile stress wave. At the same time, the loss of well-defined organelles and vacuolation of the anterior epithelial cells shown in electron micrographs is consistent with thermal damage. It is known that stress waves can decrease cell viability and increase cell permeability.[12, 13]Thus thermoelastic stress waves may not be the primary damage mechanism, but they may serve to potentiate thermal damage. If this is indeed the case, it may explain why the temperature rises associated with the damage threshold are lower than those that would be predicted by the modified critical temperature law that describes damage for longer exposures.

**Tm:YAG laser damage at 2.02 μm.**

The Tm:YAG laser was built at the Naval Research Laboratory (NRL) using APL funds. The laser was completed in 1994 prior to the beginning of this contract, but other than for its initial evaluation, it was not used until May 1998. When the laser was first received from NRL its output was ~600 mW, but by May 1998 the maximum power we were able to achieve was 350 mW. We had virtually no documentation describing details of the laser; however, we finally were able to contact Dr. Robert Stoneman who had built it (Dr. Stoneman is longer at NRL). In consultation with him, we decided that a likely problem was contamination of the output side of the crystal. Unfortunately the crystal is not accessible without totally disassembling the laser and he strongly advised us against taking this step. After consulting with our contracting officer's representative, Mr. Bruce Stuck, we decided to first use the laser with its 350 mW output. We did several calculations of temperature histories for multiple-pulse exposures which suggested that we would be able to produce threshold damage for reasonable exposure parameters.

*Tm:YAG laser characterization*

We verified the operating wavelength of the laser by measuring its output with a monochromator. It was 2.02 μm as expected. We verified that the laser was operating in the fundamental $TEM_{00}$ mode by directly viewing of the beam on a fluorescent screen and by profiling with a knife edge. The irradiance profile for a laser operating in the $TEM_{00}$ is a Gaussian given by

$$I = I_0 \exp\left[\left(-r / r_{1/e}\right)^2\right], \qquad (1)$$

where the irradiance on the beam axis, $I_0$, is equal to the total power in the beam divided by the area of the beam at the 1/e radius, $r_{1/e}$. The power measured as a knife edge is scanned in the $x$ direction across such a beam is given by [14, 15]

$$P(x) = \frac{P}{2}\left\{1 - erf\left[\frac{(x - x_0)}{r_{1/e}}\right]\right\}, \qquad (2)$$

where $x_0$ is the position of the center of the beam, $P$ is the total laser power and *erf* is the error function. We use the program Mathematica to obtain a non-linear least squares fit to Equation 2 in terms of the three parameters $r_{1/e}$, $x_0$, and $P$. The results of a typical beam scan are shown in Figure 5. The value of $\chi^2$ for the fit indicates that the beam quality is excellent.

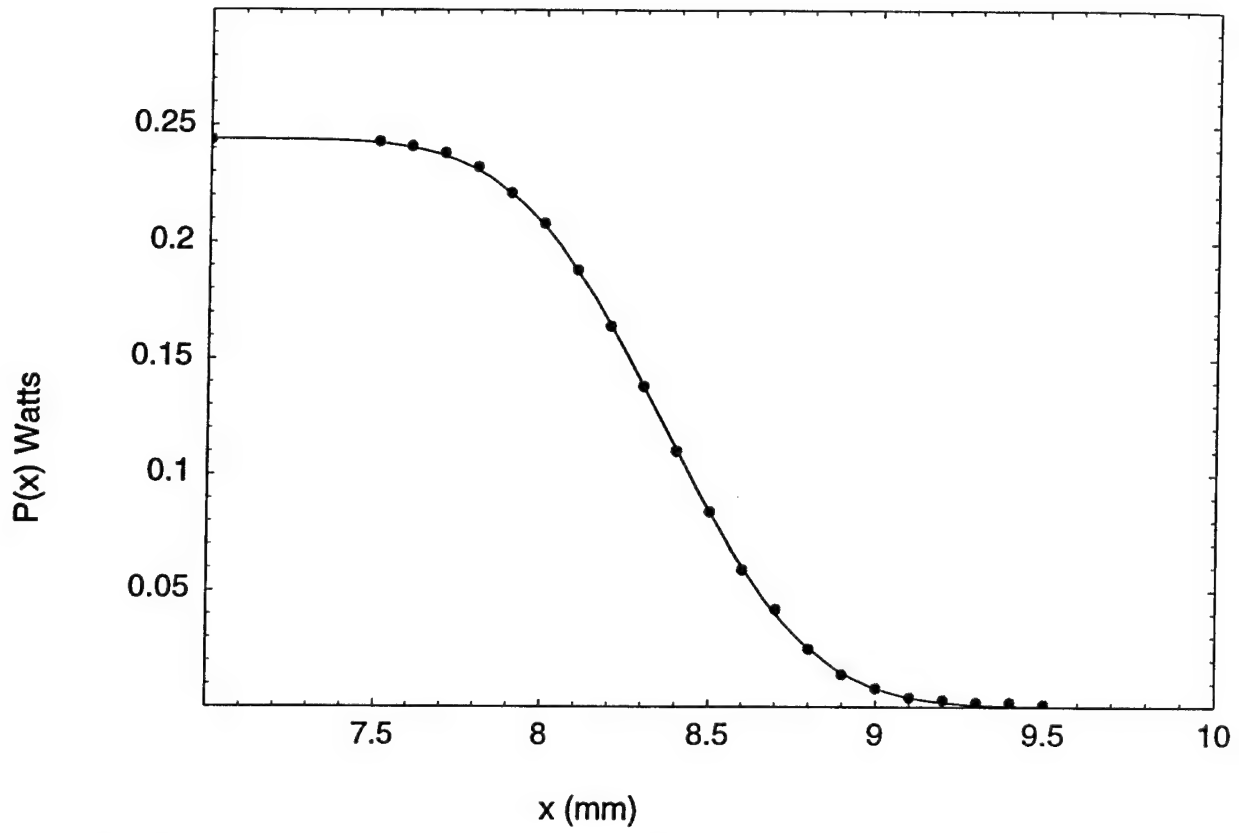


**Figure 5:** Profile of the Tm:YAG laser beam using a scanning knife edge. The solid line is a best least-squares fit to Equation 2, for which $r_{1/e}$ = 0.492 mm, $x_0$ = 8.359 mm, and $P$ = 0.244 W. For this fit, $\chi^2 = 1.7 \times 10^{-6}$, which indicates that the fit is excellent.

*Multiple-pulse damage thresholds.*

Epithelial damage thresholds were measured for sequences of pulses at frequencies of 1, 10, 20, and 100 Hz. The duration of the individual pulses was 0.300 s at 1 Hz, 0.025 s at 10 and 20 Hz, and 0.005 s at 100 Hz. The nominal 1/e diameter of the beam was 1 mm. The actual beam diameter at the position of the cornea surface was controlled with a quartz lens and was determined each experimental session by profiling with a knife edge as previously described. Exposure duration and pulse rate for the exposures at 10, 20, and 100 Hz were controlled with a Princeton Applied Research chopper. The chopper was used in conjunction with a Uniblitz shutter that acted as a gate to allow passage of the desired number of pulses. Pulse durations and pulse repetition frequencies were measured using a LeCroy 9354M digital oscilloscope. For the exposures at 1 Hz, pulse duration and frequency were controlled with the Uniblitz shutter that was calibrated using the digital oscilloscope.

Figures 6 and 7 show lesions resulting from exposures to 50 and 999 pulses at 20 Hz. The irradiances for these particular exposures were above the respective damage thresholds as noted in the captions. Lesions produced by exposures even slightly above threshold also are circular and well defined. Figure 8 shows a H and E stained section of the lesion shown in Figure 7.

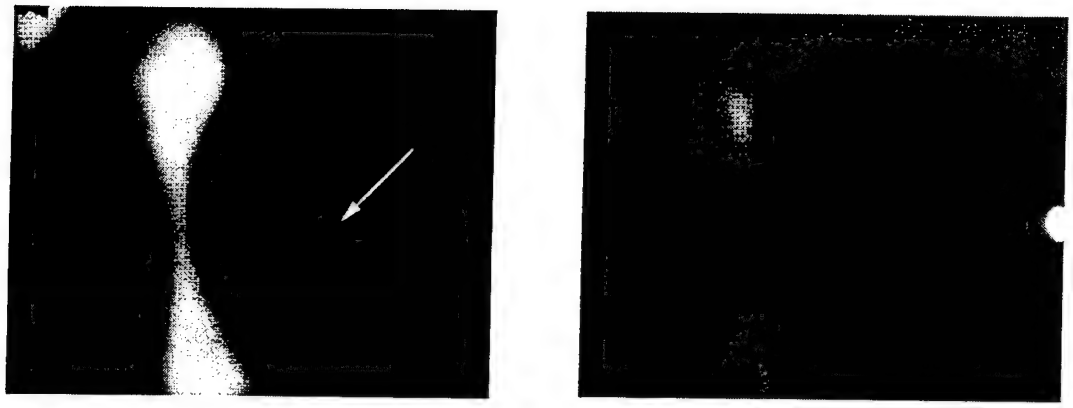

**Figure 6:** Broad- (left) and narrow-slit (right) views of a lesion (arrow in broad slit view) resulting from an exposure to 50 pulses from the Tm:YAG laser. The pulse repetition frequency was 20 Hz, the individual pulse duration was 25 ms, and the irradiance was 29.4 W/cm$^2$, which is approximately 30 percent above the damage threshold for these conditions.

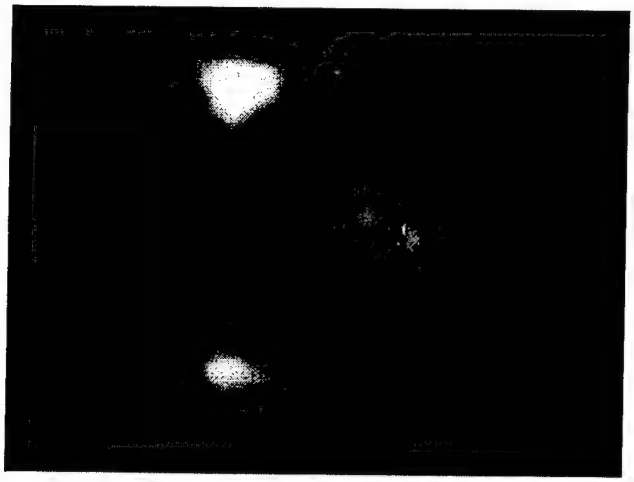

**Figure 7:** Lesion resulting from an exposure to 999 pulses from the Tm:YAG laser. The pulse repetition frequency was 20 Hz, the individual pulse duration was 0.025 s, and the irradiance was 18.9 W-cm$^{-2}$, which is approximately 70 percent above the damage threshold for these conditions.

**Figure 8:** H and E stained section of the lesion shown in Figure 7. There is loss of superficial epithelium and possible pyknosis of the remaining epithelium. There are no obvious stromal abnormalities.

The damage thresholds determined for all pulse repetition frequencies are compiled in Table 3. The threshold for a single 0.300 s pulse also was determined. As shown in Figure 9, this single-pulse threshold exposure is consistent with single-pulse damage thresholds that were determined for Tm:YAG radiation in an earlier study.[16] The threshold irradiances for the exposures at 1 Hz (0.300 s pulse duration) are plotted in Figure 10 as functions of the number of pulses. The line is a least-squares fit to a power law of the form $I_{th} = CN^{-\alpha}$, where $C = 33.8$

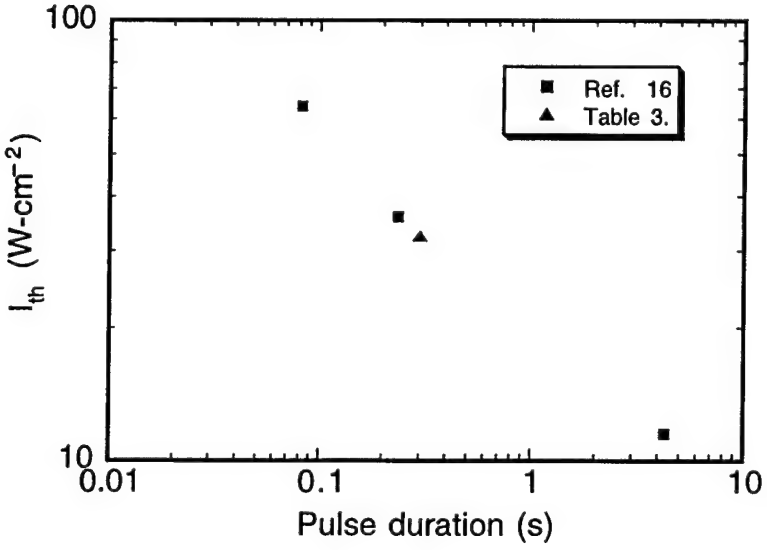


**Figure 9:** Single pulse damage thresholds for Tm:YAG laser radiation.

W/cm$^2$ and $\alpha = 0.287$ ($R = 0.997$). The value of the constant $C$ is within 4 percent of the single-pulse threshold irradiance. As will be shown below, this contrasts with the results obtained for sequences of 0.025 s pulses at frequencies of 10 and 20 Hz and sequences of 0.005 s pulses at 100 Hz.

**Table 3**: Threshold energy densities and calculated maximum temperature rises for sequences of pulses of Tm:YAG radiation at 2.02 μm.

| Number of Pulses | Repetition Frequency (Hz) | Pulse Duration (s) | $I_{th}$ (W-cm$^{-2}$) | $d_{1/e}$ (mm) | $\Delta T_{max}$ (C)[a] |
|---|---|---|---|---|---|
| 1 | | 0.300 | 32.5 | 0.938 | 51.2 |
| 8 | 1 | 0.300 | 19.6 | 0.946 | 41.4 |
| 32 | 1 | 0.300 | 12.9 | 1.040 | 29.2 |
| 128 | 1 | 0.300 | 8.04 | 1.032 | 18.8 |
| 25 | 10 | 0.025 | 35.0 | 0.936 | 34.6 |
| 50 | 10 | 0.025 | 29.2 | 0.984 | 32.3 |
| 200 | 10 | 0.025 | 19.6 | 1.010 | 24.7 |
| 999 | 10 | 0.025 | 15.5 | 1.018 | 20.9 |
| 25 | 20 | 0.025 | 25.4 | 1.016 | 38.9 |
| 50 | 20 | 0.025 | 22.2 | 0.984 | 40.5 |
| 200 | 20 | 0.025 | 17.4 | 0.972 | 38.8 |
| 999 | 20 | 0.025 | 11.2 | 0.984 | 27.8 |
| 150 | 100 | 0.005 | 26.1 | 0.974 | 39.6 |
| 200 | 100 | 0.005 | 26.0 | 0.928 | 42.4 |
| 500 | 100 | 0.005 | 19.0 | 0.934 | 36.6 |
| 999 | 100 | 0.005 | 15.5 | 1.016 | 32.4 |

[a] Calculated on the beam axis, 10 μm beneath the anterior tear surface.

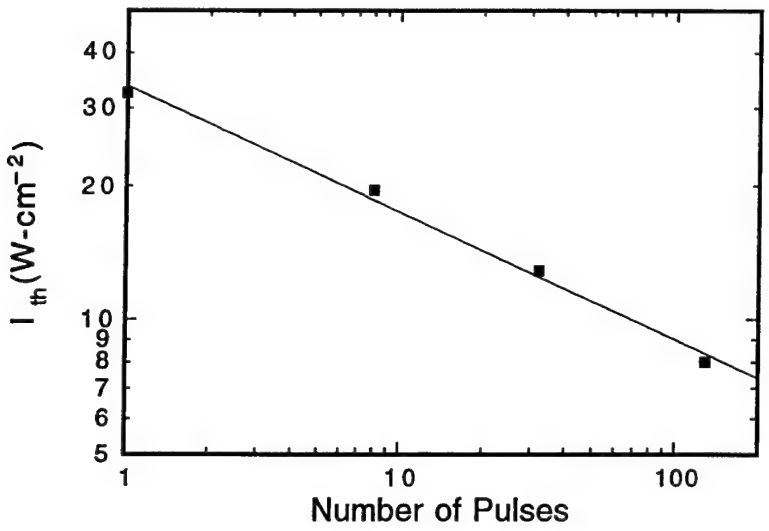


**Figure 10:** Epithelial damage thresholds for sequences of pulses from a Tm:YAG laser at a wavelength of 2.02 μm. The pulse repetition frequency was 1 Hz and the individual pulse duration was 0.300 s. The line is a least squares fit to a power law of the form $I_{th} = CN^{-\alpha}$.

The threshold irradiances for the exposures at 10, 20 and 100 Hz listed in Table 3 are plotted in Figure 11 as functions of the number of pulses. It is evident that a power law of the form $I_{th} = CN^{-\alpha}$ also can relate the data for these three exposure conditions, at least for the values of $N$ for which thresholds were measured. The straight lines are least-squares fits to this equation. The values of the parameters $C$ and $\alpha$ obtained from the fits are respectively 70.0 W/cm$^2$ and 0.22 for the thresholds at 10 Hz ($R = 0.99$), 52.7 W/cm$^2$ and 0.22 for the thresholds at 20 Hz ($R = 0.99$), and 116 W/cm$^2$ and 0.29 ($R = 0.99$) for the thresholds at 100 Hz.

As noted previously, the individual pulse durations were 0.025 s for exposures at 10 and 20 Hz, and 0.005 s for the exposures at 100 Hz. The fits for the 10 and 20 Hz exposures have the same value of the parameter $\alpha$, but have different values of the parameter $C$ (i.e., they are parallel on the log-log plot); therefore the power law relationships must break down for smaller numbers of pulses, because they do not converge to a single value for the single-pulse threshold. Indeed, the values of the parameter $C$ for both sets of data are much lower than the threshold for an exposure to a single 0.025 s pulse that would be expected on the basis of previous data and the threshold for a single 0.300 s pulse that was determined in this study.[16]. These data suggest that the single-pulse threshold for a 0.025 s exposure with a 1/e beam diameter of 1 mm would be approximately 100 W/cm$^2$ (c.f., Figure 9). Unfortunately the maximum power output of the Tm:YAG laser (~0.3 W, which includes the reflective losses at the quartz focusing lens and the attenuator) precludes obtaining thresholds with 0.025 s pulses and a 1/e beam diameter of ~1 mm for less than 25 pulses at 10 Hz and for less than about 10 pulses at 20 Hz. Similarly, the value of

$C$ for the exposures to 0.005 s pulses at 100 Hz also is lower than the single-pulse threshold that would be predicted from the data in Figure 9 (viz., ~190 W/cm$^2$). Therefore at present, we are not able to determine where the data begin to deviate from the power law dependence as the number of pulses is reduced.

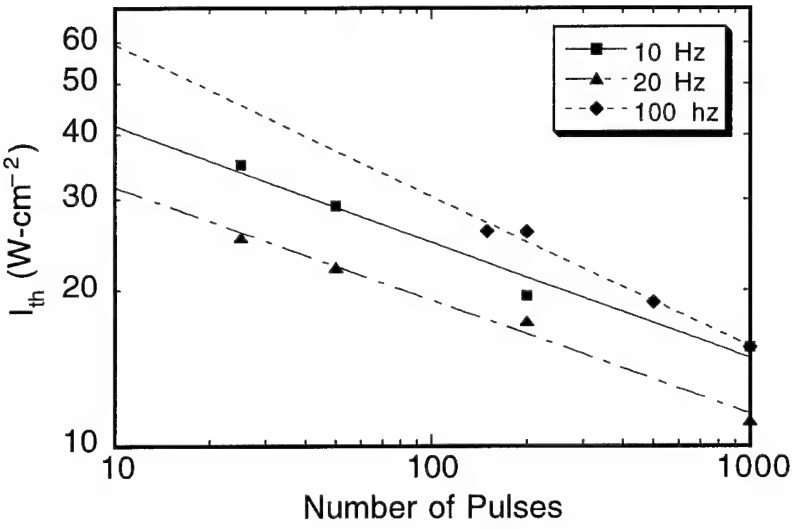


**Figure 11:** Dependence of the threshold irradiance on the number of pulses at pulse repetition frequencies of 10, 20 and 100 Hz. The lines are least-squares fits to a power law of the form $I_{th} = CN^{-\alpha}$.

A re-examination of epithelial damage threshold data for multiple-pulse $CO_2$ laser exposures to sequences of 0.001 s pulses at 20 and 100 Hz, which were determined in experiments performed under a previous contract from the Army Medical Research and Development Command, reveals similar behavior.[5, 17] These data are reproduced in Figure 12 where the measured threshold for a single 0.001 s pulse is indicated by the arrow. The lines are least-squares fits to the power law $I_{th} = CN^{-\alpha}$ for $N \geq 10$. Just as for the Tm:YAG exposures, the thresholds for the higher pulse repetition frequency lie below those for the lower frequency and the fits for $N \geq 10$ extrapolate to values that are well below the measured single-pulse threshold. On the other hand, threshold data for sequences of 0.010 s pulses at 1, 10 and 20 Hz determined during that earlier study have power law dependencies and the values of the constant $C$ for each of the exposure conditions are within ± 5 percent of the measured threshold for a single 0.010 s pulse (data not shown).

The present results for Tm:YAG damage and the previously obtained results for $CO_2$ damage from multiple pulses having individual pulse durations ≥ 0.001 s show that, for small numbers of pulses, there can be substantial deviations from the predictions of damage thresholds provided by an empirical relationship of the form $I_{th} = CN^{-\alpha}$. The deviations are such that a

relationship of this form, which accurately correlates the damage for large numbers of pulses, underestimates the damage threshold for small numbers of pulses and the single-pulse threshold.

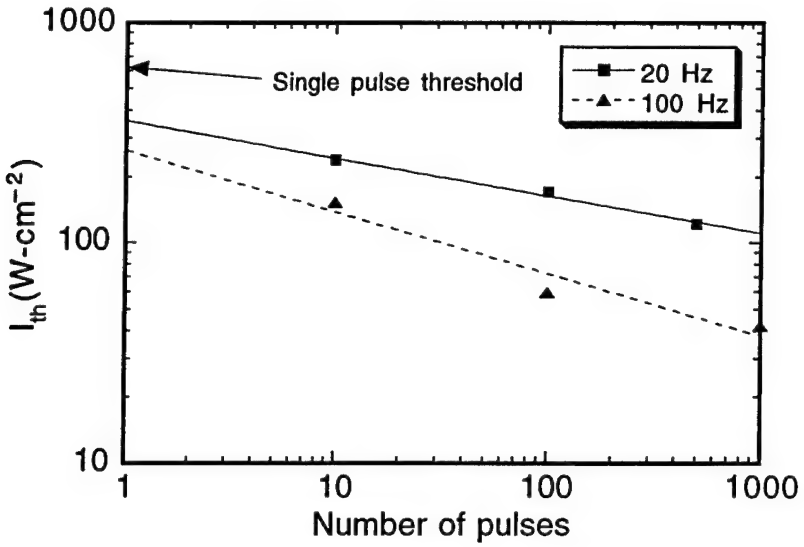


**Figure 12:** $CO_2$ laser epithelial damage thresholds taken from Reference 5. The threshold irradiance is shown as a function of the number of pulses at pulse repetition frequencies of 20 and 100 Hz. The individual pulse duration for the exposures was ~0.001 s. The lines are fits to a power law of the form $I_{th} = CN^{-\alpha}$ for $N \geq 10$. The measured threshold for a single 0.001 s pulse is indicated by the arrow.

We calculated temperature histories at a point on the beam axis, 10 μm below the anterior tear surface for the damage threshold exposures listed in Table 3. As noted previously, this location is just inside the anterior-most epithelial cells. Calculations were done as described previously.[5, 17-19] We used a value of 54.9 cm$^{-1}$ for the absorption coefficient of 2.02 μm radiation.[20] The maximum temperature increases for the various exposures are listed in Table 3. Previous studies found that epithelial damage thresholds for exposures to single pulses of Tm:YAG and $CO_2$ laser radiation were correlated by a critical temperature damage model.[5, 16, 17] For the Tm:YAG exposures, the average critical temperature increase (again calculated at a point on the beam axis, 10 μm below the anterior tear surface) was 44° C for exposure durations between 0.082 and 4.28 sec.[16] If the calculation for the single 0.300 s pulse in the present investigation is included, the average becomes 46.0 ± 5 °C (where ± denotes the full range of values). Thus the calculated maximum temperature increases for the single-pulse Tm:YAG exposures are constant to within about ± 10 percent. For the $CO_2$ laser exposures, the critical temperature increase varied slightly for exposures between 0.001 s and 10 s and was described by a modified critical temperature law given by

$$\Delta T_{crit} = 37\tau^{-0.037}, \qquad (3)$$

where $\tau$ is the exposure duration; however, for exposures between 0.01 and 1 s, the increase was nearly constant with an average value of 40 ± 2°C (mean ± SD).[5, 17] Thus the critical temperatures for the two laser wavelengths are comparable.

Similarly, studies of multiple-pulse damage thresholds for $CO_2$ laser radiation have found that damage is reasonably well correlated by a critical temperature law, but that the critical temperature depends somewhat on the duration of the individual pulses. For sequences of up to 999 pulses of $CO_2$ laser radiation at repetition frequencies from 1 to 100 Hz and individual pulse durations ≥ 0.001 s, the average calculated temperature increases were 36.5 ± 3.6 °C for pulse durations near 0.001 s, 43 ± 4.7 °C for pulse durations near 0. 01 s, and 45.3 ± 3 °C for pulse durations near 0.30 s (where ± denotes the full range of values).[5] For the experiments with 80 ns pulses that were discussed in the first section of this report, the temperature increases were 29.4±2.8 C for the exposures at 10 Hz and 32.0±2.0 C for the exposures at 16 Hz (mean ± SD). For all of these cases, the maximum temperature increases are constant to within ± 10 percent of their mean values, independent of the number of pulses. In contrast, the only Tm:YAG exposures that are in accord with this type of critical temperature model are those with 0.005 s pulses at a frequency of 100 Hz. For these experiments the average calculated temperature increase is 37.4 ± 5 °C (where ± denotes the full range of values). The calculated temperature increases for the Tm:YAG exposures with 0.025 s pulses at 10 and 20 Hz, deviate from this type of critical temperature model for sequences having greater than 50 pulses at 10 Hz and greater than 200 pulses at 20 Hz. The temperature increases calculated for the exposures with 0.300 s pulses at 1 Hz, also deviate from a critical temperature model. The reasons for these deviations from a critical temperature damage model are not yet understood.

**Retinal temperature calculations for rectangular irradiance distributions**

Retinal temperature calculations were made using methods described by Freund et al[21] and were done in close consultation with our colleagues Dr. David Sliney and Mr. Wes Marshall at CHPPM. In this initial study, the temperatures were calculated at a point on the beam axis, 4 μm in front of the pigment epithelium where the photoreceptors are located. We used an absorption coefficient for the retinal pigment epithelium that is appropriate for Argon laser radiation at 514 nm.[22] The damage threshold was determined by finding the irradiance for which the damage integral was equal to one. Parameters in the damage integral were taken from Birngruber.[22] Calculations were done for rectangular beams whose width, $2a$, and height, $2b$, varied among the values 25, 50, 100, 200, 500, 1000, 2000 μm; all possible combinations were considered. The exposure durations considered were 3 μs, 10 μs, 0.001 s, and 10 s. For each such calculation, a similar calculation was done for a beam with a Gaussian profile having the same 1/e area (i.e., $r_{1/e} = 2\sqrt{(ab/\pi)}$ ). The threshold irradiances obtained from these calculations were then used to compute the total energy predicted to cause damage and the thresholds for the rectangular and Gaussian beams were compared. For exposure durations of 3 μs, 10 μs, and 0.001 s, the total energies at the damage threshold for rectangular and Gaussian beams having equal 1/e areas were in excellent agreement. For the 10 s exposure, there was good agreement for rectangular beams with low aspect ratios; but there was only fair agreement for beams with high aspect ratios where the effects of lateral heat conduction become more important.

## Key Research Accomplishments

- Determined multiple-pulse corneal damage thresholds for 80 ns pulses of $CO_2$ laser radiation at 10.6 μm.
- Determined the first (to our knowledge) multiple-pulse corneal damage thresholds for mid-infrared laser radiation (Tm:YAG radiation at 2.02 μm).
- Demonstrated that corneal damage thresholds are correlated by an empirical power law relationship between either the energy density (per pulse) or the irradiance and the number of pulses. For some exposure conditions the law underestimates the damage threshold for small numbers of pulses.
- Demonstrated that threshold damage from sequences of 80 ns pulses of $CO_2$ laser radiation is described by a critical temperature damage model, but thermoelastic pressure waves may potentiate the thermal damage.
- Demonstrated that threshold damage from single pulses of 2.02 μm laser radiation is described by a critical temperature damage model (at least for pulse durations between approximately 0.10 and 5 s)
- Demonstrated that a critical temperature damage model sometimes, but not always, describes threshold damage from sequences of pulses of 2.02 μm laser radiation.
- Histology of lesions resulting from exposures to sequences of pulses of 2.02 μm laser radiation up to 70 percent above the damage threshold shows that damage is confined to the corneal epithelium.
- Retinal damage was modeled for rectangular irradiance profiles such as those produced by diode lasers. Predicted thresholds were compared to those for Gaussian beams having equal 1/e areas. Agreement was excellent for rectangular beams with low aspect ratios; but only fair for beams with high aspect ratios where the effects of lateral heat conduction become more important at longer exposures.

## Reportable Outcomes

### Abstracts

R. L. McCally and C. B. Bargeron, "Corneal Damage Thresholds from Sequences of Short Pulses of $CO_2$ Laser Radiation," *Invest. Ophthalmol. Vis. Sci.* **38**, S541 (1997). Presented at the Annual ARVO meeting, Ft. Lauderdale, FL.

### Conferences and Workshops

R. L. McCally and C. B. Bargeron, "Epithelial Damage Thresholds for Sequences of 80 ns Pulses of 10.6 μm Laser Radiation," in *1997 International Laser Safety Conference*, Orlando, FL, 138-143 (1997)

R. L. McCally, R. A. Farrell, C. B. Bargeron and W. R. Green, "Thermal Models of IR Laser Effects," in *Proceedings of the Infrared Lasers and Millimeter Waves Workshop, the Links Between Microwaves and Laser Optics*, Brooks AFB TX, 21-22 Jan 1997. Published as AFRL-HE-BR-PC-1999-002, February 1999. pp. 323-341.

R. L. McCally and C. B. Bargeron, "Corneal Damage Thresholds for Exposures to Tm:YAG Laser Radiation at 2.02 μm," *Second Workshop on Infrared Lasers and Millimeter Waves: Opportunities for research at the Microwave/Laser Interface*, Cloudcroft, NM, 11-13 August, 1999.

**Publications**

R. L . McCally and C. B. Bargeron, "Epithelial Damage Thresholds for Sequences of 80 ns Pulses of 10.6 μm Laser Radiation," *Jour. of Laser Appl.* **10**, 137-139 (1998).

R. L . McCally and C. B. Bargeron, "Epithelial Damage Thresholds for Multiple-Pulse Exposures to 80 ns Pulses of $CO_2$ Laser Radiation," submitted to Health Physics. Attached as Appendix B.

## Conclusions

Threshold damage to the corneal epithelium resulting from exposure to sequences of 80 ns pulses of $CO_2$ laser radiation is correlated by an empirical power law of the form $ED_{th} = CN^{-\alpha}$ in which $ED_{th}$ is the threshold energy density and $N$ is the number of pulses in the sequence. Although the empirical constants $C$ and $\alpha$ appear to differ slightly for the thresholds obtained at 10 Hz and at 16 Hz, both fits fall within the ±10 percent accuracy estimated from the bracketing procedure used to determine the thresholds. If both sets of data are assumed to be part of the same population, the empirical constants are $C = 295.5$ mJ-cm$^{-2}$ - pulse$^{-1}$ and $\alpha = 0.178$ ($R = 0.984$). The values for the constant $C$ obtained either for the individual data sets or the combined data all differ by less than 5 percent from the measured damage threshold energy density for a single pulse. Temperature calculations reveal that the maximum temperature increase on the beam axis, 10 μm beneath the anterior tear surface, resulting from the different threshold exposures is essentially constant, which suggests that the damage mechanism has a substantial thermal component and can be described by a critical temperature damage model. Additional damage determinations for multiple-pulse exposures using cooled corneas were done with the goal of clarifying the damage mechanism. The results of these experiments bolstered the view that there is a substantial thermal component to the damage, at least for sequences of pulses.

Corneal epithelial damage thresholds for multiple-pulse exposures to mid-infrared radiation from a Tm:YAG laser (wavelength 2.02 μm) are correlated by a power law of the form $I_{th} = CN^{-\alpha}$, which relates the threshold irradiance, $I_{th}$, to the number of pulses in the sequence. The constant $C$ differs depending on the pulse repetition frequency and individual pulse duration. The exponent $\alpha$ is varies between 0.22 and 0.29. For exposures to 0.300 s pulses at 1 Hz, the value of $C$ is within 4 percent of the threshold for a single pulse. Because of laser power

limitations, thresholds could not be determined for fewer than 25 pulses at pulse repetition frequencies of 10 and 20 Hz (pulse duration 0.025 s), and for fewer than 150 pulses at 100 Hz (pulse duration 0.005 s). For these exposure conditions the value of $C$ is less than the predicted threshold for single pulses having the same duration. A re-examination of multiple-pulse thresholds for $CO_2$ radiation with an individual pulse duration of 0.001 s, which also were correlated by a power law, showed that the constant $C$ also was substantially less than the single pulse threshold. In terms of setting safety standards, the power law determined for larger numbers of pulses will, at least for some exposure conditions, underestimate the damage threshold for small numbers of pulses. This would provide an additional margin of safety for small numbers of pulses. However, if one were to use the measured damage threshold for a single-pulse exposure for the value of the constant $C$, the resulting relationship would tend to overestimate the damage threshold for large numbers of pulses (i.e., it would lessen the margin of safety).

Previous studies found that epithelial damage thresholds for exposures to single-pulses of Tm:YAG radiation and to both single- and multiple-pulses of $CO_2$ radiation with pulse durations $\geq 0.001$ s also were correlated by a critical temperature damage model. In contrast, temperature calculations based on the thresholds for multiple-pulse exposures to Tm:YAG radiation show that the critical temperature damage model does not describe the data for large numbers of pulses.

Retinal damage thresholds were predicted for rectangular irradiance distributions such as those that would be produced by diode lasers. For shorter exposures (3 μs to 0.001 s), the predicted damage thresholds for rectangular beams were in excellent agreement with those for Gaussian beams having equal 1/e areas. For longer exposures (10 s), the agreement was good for rectangular beams with low aspect ratios; but it was only fair for beams with high aspect ratios where lateral heat conduction becomes an important factor.

## References

1. R. A. Farrell, R. L. McCally, C. B. Bargeron, and W. R. Green, *Structural Alterations in the Cornea from Exposure to Infrared Radiation*, U.S. Army Medical Research and Development Command, Fort Detrick, MD 21701-5012 (1989).

2. R. A. Farrell, C. B. Bargeron, R. L. McCally, and W. R. Green, "Corneal Effects Produced by IR Laser Radiation," in *Laser Safety, Eyesafe Laser Systems, and Laser Eye Protection*, Los Angeles, California, 59-70 (1990).

3. R. L . McCally and C. B. Bargeron, "Epithelial Damage Thresholds for Sequences of 80 ns Pulses of 10.6 μm Laser Radiation," in *1997 International Laser Safety Conference*, Orlando, FL, 138-143 (1997).

4. R. L . McCally and C. B. Bargeron, "Epithelial Damage Thresholds for Sequences of 80 ns Pulses of 10.6 μm Laser Radiation," *Jour. of Laser Appl.* **10**, 137-139 (1998).

5. C. B. Bargeron, O. J. Deters, R. A. Farrell, and R. L. McCally, "Epithelial Damage in Rabbit Corneas Exposed to $CO_2$ Laser Radiation," *Health Phys.* **56**, 85-95 (1989).

6. D. L. Lund, B. E. Stuck, and E. A. Beatrice, *Biological Research in Support of Project MILES. Presidio of San Franscisco, CA*, Letterman Army Institute of Research 96 (1981).

7. D. M. Maurice, *The Cornea and Sclera*, in *The Eye,* H. Davson (ed.), Academic Press, Orlando, 1-158 (1984).

8. R. F. Rosenbluth and I. Fatt, "Temperature Measurements in the Eye," *Exp. Eye Res.* **25**, 325-341 (1977).

9. M. W. Sigrist and F. K. Kneubühl, "Laser Generated Stress Waves in Solids," *J. Acoust. Soc. Am.* **64**, 1652-1663 (1978).

10. Y. H. Berthelot and I. J. Busch-Vishniac, "Laser-induced Thermoacoustic Radiation," *J. Acoust. Soc. Am.* **78**, 2074-2082 (1985).

11. R. O. Esenaliev, A. A. Oraevsky, V. S. Letokov, A. A. Karabutov, and T. V. Malinsky, "Studies of Acoustical and Shock Waves in the Pulsed Laser Ablation of Biotissue," *Lasers in Surg. Med.* **13**, 470-484 (1993).

12. A. G. Doukas and T. J. Flotte, "Physical Characteristics and Biological Effects of Laser-induced Stress Waves," *Ultrasound in med. and Biol.* **22**, 151-164 (1996).

13. S. Lee, T. Anderson, H. Zhang, T. J. Flotte, and A. G. Doukas, "Alteration of Cell membrane by Stress Waves *In Vitro*," *Ultrasound in Med. and Biol.* **22**, 1285-1293 (1996).

14. J. M. Khosrofian and B. A. Garetz, "Measurement of a Gaussian Laser Beam Diameter by the Direct Inversion of Knife-edge Data," *Appl. Optics* **22**, 3406-3410 (1983).

15. R. L. McCally, "Measurement of Gaussian Beam Parameters," *Appl. Optics* **23**, 2227 (1984).

16. R. L. McCally, R. A. Farrell, and C. B. Bargeron, "Cornea Epithelial Damage Thresholds in Rabbits Exposed to Tm:YAG Laser Radiation at 2.02 μm," *Lasers in Surgery and Medicine* **12**, 598-603 (1992).

17. R. A. Farrell, R. L. McCally, C. B. Bargeron, and W. R. Green, "Structural Alterations in the Cornea from Exposure to Infrared Radiation," *JHU/APL TG 1364* (1985).

18. C. B. Bargeron, R. L. McCally, and R. A. Farrell, "Calculated and Measured Endothelial Temperature Histories of Excised Rabbit Corneas Exposed to Infrared Radiation," *Exp Eye Res.* **32**, 241-250 (1981).

19. C. B. Bargeron, R. A. Farrell, W. R. Green, and R. L. McCally, "Corneal Damage from Exposure to IR Radiation: Rabbit Endothelial Damage Thresholds," *Health Phys.* **40**, 855-862 (1981).

20. E. F. Maher, *Transmission and Absorption Coefficients for Ocular Media of the rhesus monkey*, USAF School of Aerospace Medicine, Brooks AFB SAM-TR-78-32 (1978).

21. D. E. Freund, R. L. McCally, R. A. Farrell, and D. H. Sliney, "A Theoretical Comparison of Retinal Temperature Changes Resulting from Exposure to Rectangular and Gaussian Beams," *Lasers in the Life Sci.* **7**, 71-89 (1996).

22. R. Birngruber, F. Hillenkamp, and V. P Gabel, "Theoretical Investigations of Laser Thermal Retinal Injury," *Health Physics* **48**, 781-796 (1985).

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# Appendices

# Epithelial damage thresholds for sequences of 80 ns pulses of 10.6 $\mu$m laser radiation

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Infrared radiation from a $CO_2$ laser operating at 10.6 $\mu$m is almost totally absorbed by the tear film and corneal epithelium. Epithelial damage thresholds for single and multiple pulse exposures have been well characterized for exposures having pulse durations $\geq$1 ms and a few damage thresholds have been reported for much shorter duration pulses. However until recently, no studies have been done to determine damage thresholds from sequences of very short pulses. The purpose of this study is to begin to fill this gap. New Zealand white rabbit corneas were exposed to sequences of 80 ns pulses from a $CO_2$-TEA laser operating in the $(TEM)_{00}$ mode (Gaussian beam profile). The damage threshold was defined as the energy density, $ED_{th}$, that produced minimal superficial epithelial lesions. Thresholds have been determined for sequences of 2, 8, 32, 128, and 1024 pulses at a rate of 10 Hz. The damage data are shown to be correlated by empirical laws relating either the threshold energy density per pulse to the number of pulses, or the total threshold energy density to the duration of the pulse train. Research is underway to obtain data for pulse sequences at greater pulse rates and to examine the relative importance of thermal and acoustic damage mechanisms. © *1998 Laser Institute of America.* [S1042-346X(98)00803-1]

Key words: lasers, epithelial damage thresholds, eye safety

## I. INTRODUCTION

Infrared radiation from a $CO_2$ laser at a wavelength of 10.6 $\mu$m is strongly absorbed by the water in biological tissue. Indeed when this radiation impinges on the eye, 99% is absorbed in the first 50 $\mu$m of the cornea which includes the tear film and the epithelial cell layer. This energy is rapidly converted to heat that initially is confined to the volume of absorption and subsequently is conducted to deeper layers. This deposition of heat can lead to thermal damage. Our previous investigations of the effects of $CO_2$ laser radiation on the cornea showed that threshold damage for exposures ranging in duration from 0.001 to 10 s could be explained by a thermal damage model.[1–3] Also, epithelial damage from sequences of sub-threshold pulses is thermal in nature. The multiple-pulse exposures that were investigated consisted of pulse trains of up to 999 pulses, with pulse repetition frequencies between 1 and 100 Hz and individual pulse durations between 0.001 and 0.5 s. The temperature increase required to inflict threshold damage with sequences of such pulses is essentially the same as for a single pulse.[3] Damage from multiple pulse exposures also can be correlated by several empirical laws including the law, $ED_{th\text{-}total}=CN^{3/4}$, where $ED_{th\text{-}total}$ is the total threshold energy density in J/cm$^2$, $N$ is the number of pulses, and the constant $C$ depends on pulse repetition frequency.[3]

There is only limited corneal damage data for exposures to very short pulses[4–7] and only some very preliminary measurements for multiple pulse exposures.[6,7] For very short pulses, the induced temperature gradient in the material can lead to the generation of thermoacoustic pulses that propagate into the tissue. Earlier histological investigations using light and electron micrographs of corneas exposed to a single 80 ns pulse from a $CO_2$-TEA laser just above the damage threshold revealed unusual disruptions of the anterior epithelial surface for threshold exposures. The characteristics of these disruptions differed from those observed with simple thermal damage at longer pulse durations and have features that appeared to be consistent both with a thermal mechanism and with a mechanical (e.g., acoustic) damage mechanism.[6,7] Health policy makers in the civilian and defense sectors require additional damage data for short pulse exposures. In this article we describe new damage threshold determinations for sequences of 80 ns pulses from a $CO_2$-TEA laser.

## II. METHODS

### A. Research animals and their handling

Healthy New Zealand white rabbits of either sex weighing 2.5–3.5 kg were used for the experiments. The rabbits were treated in accordance with the Association for Research in Vision and Ophthalmology Resolution for the Use of Animals in Research. Prior to exposure the rabbits were anesthetized with an intramuscular injection of xylazine and ketamine hydrochloride (Rompun–Ketaset) in the proportions: 60% of 20 mg/ml Rompun to 40% of 100 mg/ml Ketaset by volume. In addition a topical anesthesia (proparacaine hydro-

 

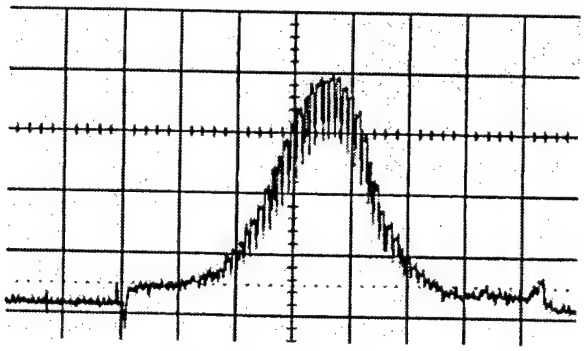

FIG. 1. Typical beam profile of the $CO_2$-TEA laser. The center-to-center spacing of adjacent elements of the Spiricon detector is 0.2 mm. The $1/e$ radius of the beam is 1.86 mm.

chloride) and Atropine 1% was applied to each eye before exposure. The anesthetized animals were placed in a conventional holder for exposure. A speculum was inserted in the eye about 1 min before exposure and the eye was irrigated with BSS solution (Alcon Surgical); however, in order to create a reproducible "tear film," the irrigation was stopped 20 s before the exposure and excess fluid was blotted at the limbus. Animals were sacrificed by an intravenous injection of sodium pentobarbital and the corneas inspected for damage as described below.

### B. Damage determinations

The criterion that we have used for minimal epithelial damage is that due to Brownell and Stuck,[8] namely the presence of a superficial gray-white spot that develops within 1/2 h after exposure. Corneas are assessed for damage by slit-lamp examination. We have found that the damage threshold is sharply defined; i.e., only rarely is there overlap between exposures that produce minimal lesions and those that do not. Therefore we do not use statistical procedures such as probit analysis in order to determine the threshold, as these would require the use of more animals than we deem necessary. We make one exposure per eye, bracketing exposures above and below threshold. The bracket is narrowed until there is only about a 10% difference in energy. The threshold is then taken to be at the center of the bracket. In our experience a single such determination requires an average of eight eyes (four rabbits).

### C. Laser systems

The exposures were made with a Boston Laser (Model 220S) $CO_2$-TEA laser. This laser delivers 80 ns pulses at pulse repetition frequencies up to ~20 Hz. The laser is operated in the $TEM_{00}$ mode (Gaussian beam profile) for all exposures. In the $TEM_{00}$ mode, the peak energy density $ED_0$ is related to the total energy in the beam by $ED_0 = E/A$, where $A$ is the area within the $1/e$ beam radius; i.e., the radius at which the energy is $1/e$ or 36.8% of its peak value on the beam axis. The mode quality is verified and the $1/e$ beam radius is measured using a Spiricon 64 element linear pyroelectric array detector before and after each exposure. Pulse energy also is measured before and after each exposure with a Scientec thermal detector. Figure 1 shows a typical beam profile for which the measured $1/e$ radius is 1.86 mm.

TABLE I. Epithelial damage thresholds for sequences of 80 ns pulses of 10.6 $\mu$m radiation. The values of $ED_{th}$ are the peak energy density of the Gaussian profile at the threshold exposure.

| Number of pulses | $ED_{th}$(mJ/cm$^2$/pulse) |
|---|---|
| 1 | 307 |
| 2 | 235 |
| 8 | 228 |
| 32 | 154 |
| 128 | 136 |
| 1024 | 95 |

## III. RESULTS AND DISCUSSION

Epithelial damage thresholds were determined for sequences of 1, 2, 8, 32, 128, and 1024 pulses at a pulse repetition frequency of 10 Hz. The results are summarized in Table I. The data for 1 and 2 pulses are refinements of preliminary results published earlier.[6,7]

As noted in Sec. I, we showed in an earlier study that epithelial damage produced by sequences of longer duration pulses ($\geq$1 ms) could be correlated by an empirical relation in which the threshold energy density per pulse was proportional to the $-1/4$ power of the number of pulses.[3] Coincidentally, this same relation holds for *retinal* damage from multiple pulse exposures.[9] Figure 2 shows a plot of the data in Table I. The least squares fit shows that these results are also correlated by a power law of the form:

$$ED_{th} = 291N^{-0.16}, \quad (1)$$

with a correlation coefficient $R = 0.98$. Thus, with the short 80 ns pulses, there is a weaker dependence on the number of pulses than for the longer duration pulses.

Figure 3 shows a different empirical correlation of the data in Table I which holds for exposure durations greater than 0.1 s (in this case for $N > 2$). The *total* threshold energy density in the pulse sequence, $ED_{th\text{-}total}$, is related to the duration of the sequence by a power law

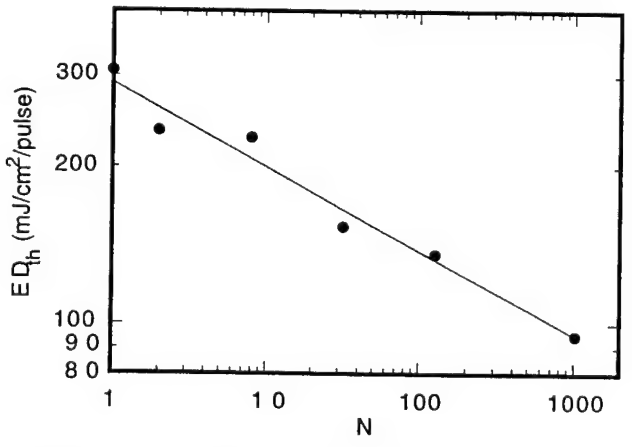


FIG. 2. Dependence of the threshold energy density per pulse on the number of pulses. A least squares fit shows that the experimental data are described by Eq. (1).

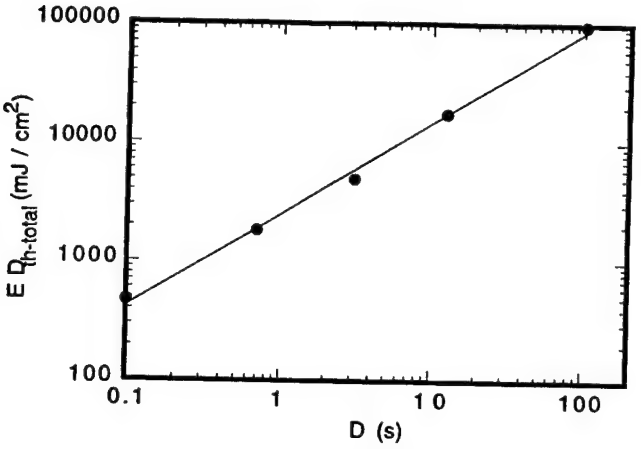


FIG. 3. Total threshold energy density for the data in Table I plotted as a function of the duration of the pulse train.

$$ED_{th\text{-}total}=2472D^{0.771},$$

with a correlation coefficient of 0.9997. In this equation, the duration is given by $D=\tau+(N-1)/f$, where $\tau$ is the duration of the individual pulses and $f$ is the pulse repetition frequency. In the earlier study involving pulses with durations $t\geqslant 1$ ms, the total threshold energy density was proportional to the 2/3 power of the exposure duration.[3]

The empirical relationships that correlate the multiple-pulse damage data encourage us to seek physical models that will explain them. We are presently in the process of calculating temperature histories for the exposure conditions listed in Table I in order to determine if an approximate critical temperature damage model similar to the one that we found in the earlier study applies to these exposures.[3] Preliminary calculations for sequences of 1, 2, and 8 pulses reported in Refs. 6 and 3 suggest that the maximum temperature rises on the beam axis 10 $\mu$m beneath the anterior tear surface are somewhat lower than those found for the longer duration pulses. Furthermore, as noted in Sec. I, earlier histology studies showed evidence for acoustic as well as thermal damage.

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[1]C. B. Bargeron, R. A. Farrell, W. R. Green, and R. L. McCally, Health Phys. **40**, 855–862 (1981).
[2]R. A. Farrell, R. L. McCally, C. B. Bargeron, and W. R. Green, Johns Hopkins University Applied Physics Laboratory Technical Memorandum TG 1364 (1985).
[3]C. B. Bargeron, O. J. Deters, R. A. Farrell, and R. L. McCally, Health Phys. **56**, 85–95 (1989).
[4]H. A. Mueller and W. J. Ham, in "The Ocular Effects of Single Pulses of 10.6 $\mu$m and 2.5–3.0 $\mu$m Q-Switched Laser Radiation," Report to Los Alamos Scientific Laboratory, L Division (1976).
[5]J. A. Zuclich, M. F. Blankenstein, S. J. Thomas, and R. F. Harrison, Health Phys. **47**, 829–835 (1984).
[6]R. A. Farrell, R. L. McCally, C. B. Bargeron, and W. R. Green, "Structural Alterations in the Cornea from Exposure to Infrared Radiation," U.S. Army Medical Research and Development Command, Fort Detrick, MD 211701-5012 Final Report (1989).
[7]R. A. Farrell, C. B. Bargeron, R. L. McCally, and W. R. Green, in *Corneal Effects Produced by IR Laser Radiation*, SPIE Proceedings (SPIE, Bellingham, WA, 1990), Vol. 1207, pp. 59–70.
[8]A. S. Brownell and B. E. Stuck, Proceedings of the 9th Army Science Conference Vol. 1, pp. 123–138.
[9]D. L. Lund, B. E. Stuck, and E. A. Beatrice, in *Biological Research in Support of Project MILES. Presidio of San Francisco, CA* (Letterman Army Institute of Research, San Francisco, 1981).

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**Appendix B**
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# Epithelial Damage Thresholds for Multiple-Pulse Exposures to 80 ns Pulses of $CO_2$ Laser Radiation*

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## Abstract

Corneal epithelial damage thresholds for exposures to 80 ns pulses of 10.6 μm infrared radiation produced by a $CO_2$-TEA laser were investigated. Thresholds were determined for sequences of 1, 2, 8, 32, 128, and 1024 pulses at pulse repetition frequencies of 10 and 16 Hz. Threshold damage is correlated by an empirical power law of the form $ED_{th} = CN^{-\alpha}$, in which $ED_{th}$ is the threshold energy density per pulse, and $N$ is the number of pulses. The constants $C$ and $\alpha$ are similar for the two pulse repetition frequencies. For the combined data set, $C = 2955$ J-m$^{-2}$-pulse$^{-1}$ (295.5 mJ-cm$^{-2}$ - pulse$^{-1}$) and $\alpha = 0.178$. This value of the constant $C$ is within 5% of the measured damage threshold for a single 80 ns pulse exposure. Temperature calculations reveal that the maximum temperature increase on the beam axis, 10 μm beneath the anterior tear surface, resulting from the different threshold exposures is essentially constant. This result is consistent with a critical temperature damage model. Damage threshold measurements on cooled corneas indicate that the damage mechanism indeed has a substantial thermal component.

## Introduction

Radiation from $CO_2$ lasers incident on the eye is almost entirely absorbed within the tear film and corneal epithelium. The absorbed radiation heats the tissue and, if it is sufficiently intense, causes damage. For exposures to single pulses lasting ≥ 1 ms, corneal damage thresholds are well documented. (Peabody et al, 1969; Peppers et al, 1969; Brownell and Stuck, 1974; Egbert

*In conducting the research described in this report, the investigators adhered to the "*Guide for Care and Use of Laboratory Animals*" prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council.
The views, opinions and/or findings contained in this report are those of the authors and should not be construed as an official Department of the Army position, policy, or decision unless so designated by other documentation.

and Maher, 1977; Bargeron et al, 1989) Damage in this regime is thermal and thresholds are correlated by either a modified critical temperature model or by a damage integral model. (Brownell and Stuck, 1974; Egbert and Maher, 1977; Bargeron et al, 1989) However, it was not possible to correlate damage from exposures to sequences of pulses having individual pulse durations $\geq$ 1 ms using the damage integral model. (Bargeron et al, 1989) Nevertheless thresholds for such exposures could be correlated using a critical temperature model, as well as an empirical power law relationship between the threshold energy density and the number of pulses in the sequence. (Bargeron et al, 1989)

Only a few studies have been done to determine corneal damage thresholds for $CO_2$ laser exposures having pulse durations shorter than 1 ms and only very limited data for sequences of short pulses has been published. Ham and Mueller (1976) examined damage from single 1.4 ns $CO_2$ laser pulses and obtained a damage threshold far too low to be consistent with a thermal damage mechanism. Zuclich et al (1984) determined damage thresholds for single $CO_2$ laser pulses having durations of 1.7, 25, and 250 ns and stated that their results were in accord with a thermal damage mechanism. Farrell et al (1989; 1990) reported preliminary damage thresholds for a single 80 ns pulse of $CO_2$ laser radiation and for sequences of two 80 ns pulses at 1 Hz, and sequences of two and eight 80 ns pulses at 10 Hz. The temperature increase calculated for their single-pulse threshold exposure (viz., 35.5 C) was lower than that calculated for threshold exposures having durations $\geq$ 1 ms. This is counter to predictions of the modified critical temperature model that successfully correlates damage thresholds for exposures having pulse durations greater than 1 ms. (Egbert and Mayer, 1977; Bargeron et al, 1989) In that model, larger temperature increases are required to produce threshold damage for short pulses than are required for longer pulses. In addition, histology of near-threshold lesions produced by a single 80 ns pulse showed features consistent with both thermal and mechanical damage to superficial epithelial cells. Farrell et al speculated that the large temperature gradients at the anterior surface resulting from these short exposures could produce thermoelastic pressure transients that might cause mechanical damage to the superficial cells. (Sigrist and Kneubühl, 1978) Because of these observations it has not yet been possible to ascribe damage from the 80 ns pulses to either a purely thermal model or to a purely mechanical model.

More recently the damage threshold for a single 80 ns pulse of $CO_2$ laser radiation was refined and additional thresholds for sequences of 80 ns pulses at 10 Hz were reported. (McCally and Bargeron, 1998) The data for sequences of pulses were correlated by an empirical law relating the energy density per pulse to the number of pulses. Although no temperature calculations were presented, the refined single-pulse threshold is about 15 percent lower than the preliminary value that was reported previously. Thus the maximum temperature increase would be lower by the same percentage.

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It is important to assess the health effects and hazards of laser systems that emit short pulses in order to provide a rational basis for setting exposure limits to prevent ocular injury. In this paper we present additional threshold data for sequences of 80 ns pulses at a frequency of 16 Hz. These new data, together with the previously reported data at 10 Hz, are discussed in terms of a critical temperature model. The new data, like the previously reported data at 10 Hz, can also be correlated by an empirical power law relationship between the number of pulses and the energy density per pulse. In an attempt to clarify the damage mechanism, we also performed a series of experiments on corneas that had been cooled prior to exposure. The results suggest that there is a substantial thermal component to the damage, at least for sequences of pulses.

## Materials and Methods

*Laser System*

Exposures were made with a Boston Laser (Model 220S) $CO_2$-TEA[†] laser operating in the $TEM_{00}$ mode as described previously. (Farrell et al, 1989; Farrell et al, 1990; McCally and Bargeron, 1998) In the $TEM_{00}$ mode the output beam has a Gaussian irradiance profile. The laser delivers 80 ns pulses at repetition frequencies up to 16 Hz. The pulse energy was measured with a Scientech[‡] detector immediately before and immediately after each exposure. Mode quality was verified and the $1/e$ beam diameter was measured before and after each exposure using a Spiricon[§] linear pyroelectric array. The Spiricon detector array has 64 elements on 200 μm centers. It was mounted on a vertical micropositioner and was read out with a LeCroy[‖] digital oscilloscope. (McCally and Bargeron, 1998) The peak energy density on the axis of a beam with a Gaussian profile is related to the total energy in the beam, $E$, by $ED_0 = E / A$, where $A$ is the area within the $1/e$ diameter. (Note that some authors use the $1/e^2$ diameter or radius to characterize the beam. In this case, $ED_0 = 2E / A'$, where $A'$ is the area within the $1/ e^2$ diameter.) A similar relationship holds between the peak irradiance on the axis of a beam with a Gaussian irradiance profile, $I_0$, and the total power, $P$, in the beam.

*Animals*

New Zealand white rabbits of either sex weighing 1.8 – 2.3 kg were used for the experiments. The rabbits were anesthetized with an intramuscular injection of a mixture of xylazine (12 mg/kg) and ketamine hydrochloride (40 mg/kg). A topical anesthesia (proparacaine hydrochloride 1/2%) also was applied to each eye and a drop of homatropine bromide 5% was instilled to dilate the pupil. Homatropine facilitates examining the exposed corneas for minimal

[†] Boston Laser Technology Inc., 10 Commerce Way, Norton, MA 02766

[‡] Scientec model 362 Power Energy Meter, Scientec Inc., 5469 Arapahoe Ave., Boulder, CO 80303

[§] Spriacon Inc., 2600 N. Main St., Logan, UT 84321

[‖] LeCroy Model 5354M, LeCroy Corp., 700 Chestnut Ridge Road, Chestnut Ridge, NY 10977

lesions. The anesthetized animals were placed in a conventional holder where they were positioned with the aid of a low-power He-Ne laser whose beam was aligned to be coaxial with the $CO_2$ laser beam. The eyes were positioned so that incident beam from the $CO_2$ laser would be perpendicular to the cornea. A speculum was inserted in the eye about one minute prior to exposure. In order to create a reproducible tear film, the eye was irrigated with a small amount of physiological saline solution (BSS - Alcon Surgical[¶]) that was at room temperature. Irrigation was stopped about 20 s before exposure and the excess fluid was blotted at the limbus. The corneal surface was assumed to have returned to its normal temperature at the time of exposure. One-half hour after exposure, the rabbits, still under anesthesia, were sacrificed with Beuthanasia-D[#] (100 mg/kg) administered in an ear vein. The eyes were enucleated and immediately examined for damage using a Nikon photo slit-lamp[**].

In a few experiments designed to investigate the damage mechanism, we exposed enucleated eyes that were at room temperature. It was shown previously that reliable damage thresholds can be determined in freshly enucleated eyes. (McCally et al, 1992) For these experiments the rabbits were anesthetized and given a topical anesthesia and the pupils dilated exactly as was done for the *in vivo* exposures. The rabbits were then sacrificed and their eyes enucleated. The enucleated eyes were placed in BSS solution at room temperature (about 21 °C) and allowed to equilibrate for at least 5 minutes. They were then exposed using the same protocol as for the *in vivo* exposures. After exposure the eyes were placed back in the solution for 1/2 hour before examining them for damage.

*Damage Determination*

The criterion used to determine minimal epithelial damage was the presence of a superficial, barely visible, gray-white spot that develops within 1/2 hour after exposure. (Brownell and Stuck, 1974) In these experiments the damage threshold is well defined. Only rarely is there any overlap between exposures that produce minimal lesions and those that do not. Therefore statistical procedures such as probit analysis were not used to determine the threshold, as these would require using more animals than necessary. (Bargeron et al, 1989; Farrell et al, 1989; McCally and Bargeron, 1998) One exposure was made per eye, initially attempting to find broadly bracketing exposures above and below threshold. The bracket was then narrowed until there was only about a 10% difference in energy between an exposure that produced a minimal lesion and one that did not. The threshold exposure was taken to be at the center of the bracket.

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[¶] Alcon Laboratories Inc., Fort Worth, TX 76134

[#] Schering-Plough Animal Health Corp.,Kenilworth, NJ 07033

[**] Nikon model FS-3, Nikon Inc. Instrument Division, 623 Stewart Ave., Garden City, NY 11530

*Temperature calculations*

Temperature calculations were based on a Green's function solution to the heat conduction equation for an incident beam with a Gaussian irradiance profile that is absorbed according to Beer's law. (Morse and Feshbach, 1953; Carslaw and Jaeger, 1959; Chang and Dedrick, 1969; Bargeron et al, 1981a,b) The beam was assumed to impinge on a semi-infinite slab and to have constant peak irradiance for the duration of the exposure. It was also assumed that conduction was the only mode of heat transfer and that no heat was lost to the air at the epithelial interface by conduction, convection, or re-radiation. (Bargeron et al, 1989) The absorption coefficient and thermal properties of the cornea were assumed to be those of water. (Maher, 1978) The solution $T(r,z,t)$, where $r$ is the radial distance from the beam axis, $z$ is the depth into the cornea, and $t$ is time, has the form of a definite integral that can be evaluated numerically.

## Results and Discussion

Epithelial damage thresholds were determined for sequences of 2, 8, 32, 128, and 1024 pulses at a pulse repetition frequency of 16 Hz. The threshold energy densities are compiled in Table 1. For completeness, the thresholds obtained previously for pulse sequences at 10 Hz and the refined single pulse threshold (McCally and Bargeron, 1998) are also listed in the Table. Fig. 1 shows a laser lesion caused by an exposure to 128 pulses at 16 Hz. The energy density for this exposure was 1440 J-m$^{-2}$ (144 mJ-cm$^{-2}$) which is 30% greater than the damage threshold. Damage is confined to the epithelium.

The damage threshold energy densities per pulse listed in Table 1 are plotted in Fig. 2 as a function of the number of pulses. Least squares fits to these data show that the thresholds at 10 Hz and 16 Hz are both correlated by an empirical power law of the form

$$ED_{th} = CN^{-\alpha}, \qquad (1)$$

in which $N$ is the number of pulses in the sequence. The empirical constants $C$ and $\alpha$ are determined by a least-squares fit to the experimental data. For the 10 Hz thresholds, $C$ = 2190 J-m$^{-2}$- pulse$^{-1}$ (291 mJ-cm$^{-2}$-pulse$^{-1}$) and $\alpha$ = 0.162 (R = 0.976), and for the 16 Hz thresholds, $C$ = 3000 J-m$^{-2}$-pulse$^{-1}$ (300 mJ-cm$^{-2}$-pulse$^{-1}$) and $\alpha$ = 0.194 (R = 0.997). It is not possible to discern if the slight difference between the two fits is real; but, in any case, both fits fall within the ±10 percent accuracy estimated from the bracketing procedure used to determine the thresholds. If both sets of data are assumed to be part of the same population, the fitting procedure shows that $C$ = 2955 J-m$^{-2}$-pulse$^{-1}$ (295.5 mJ-cm$^{-2}$- pulse$^{-1}$) and $\alpha$ = 0.178 (R = 0.984). The values for the constant $C$ all differ by less than 5% from the measured damage threshold energy density for a

single pulse. This is well within the estimate accuracy of the procedure used for determining individual thresholds.

The power law is of the same form that we found previously for sequences of pulses having individual pulse durations between 0.001 and 1 s with pulse repetition frequencies between 1 and 100 Hz. However, for the longer pulse durations, the exponent $\alpha$ was ~ 0.25 and the coefficient depended on the duration of the individual pulses. (Bargeron et al, 1989) Coincidentally, retinal damage thresholds for sequences of pulses are also described by a power law of this same form with $\alpha = 0.25$. (Lund et al, 1981)

The maximum temperature increases calculated for the damage threshold exposures are also listed in Table 1. Temperatures are calculated at a position on the beam axis, 10μm beneath the surface of the tear layer. Thus, assuming that the tear layer is about 7 μm thick, (Maurice, 1984) the temperature increases are those that occur just inside the anterior-most epithelial cells. Because of heat conduction, the temperature at this position does not reach its maximum value until about 164 μs after the final pulse. Fig. 3 shows two illustrative temperature histories calculated for damage threshold exposures for sequences of 32 and 128 pulses at a pulse repetition frequency of 16 Hz.

The maximum temperature increases calculated for both exposure conditions (viz., 10 and 16 Hz) are constant to within ± 10 percent of their mean values, independent of the number of pulses. For the exposures at 10 Hz, $\Delta T_{max}$ = 29.4±2.8 C (mean±SD), and for the exposures at 16 Hz, $\Delta T_{max}$ = 32.0±2.0 C. The maximum temperature increases for the two exposure conditions are shown graphically in Fig. 4. The fact that the calculated maximum temperature increases are essentially constant for all of damage thresholds at a given frequency suggests that the damage mechanism has a substantial thermal component and can be described by a critical temperature damage model.

In order to elucidate the damage mechanism further, a series of damage experiments was performed on enucleated eyes that were cooled to room temperature (average 21 C) as described in Materials and Methods. The underlying hypothesis for this test is that if a critical temperature model is valid, then damage should occur for exposures that result in the same final critical temperature (not temperature increase). Thus for a cornea initially at room temperature sufficient additional energy would have to be supplied, first to raise the temperature to the *in vivo* temperature, and then to the final "damage" temperature. In a preliminary experiment using 8 pulses at 16 Hz, corneas in cooled enucleated eyes did not incur damage at the same (slightly above threshold) exposure that produced lesions in corneas *in vivo*. This finding suggested that the damage mechanism was at least partially thermal. Subsequently, damage thresholds for cooled corneas were determined for exposures to sequences of 8 and 32 pulses at 16 Hz. The threshold energy densities and the calculated maximum temperature rises for these experiments are listed in Table 2. If one assumes that the temperature of the anterior surface of a cornea *in*

*vivo* is 35 C, (Rosenbluth and Fatt, 1977) the "damage temperatures" from Table 1 for the 8 and 32 pulse 16 Hz thresholds would be respectively 339.5 K and 341.1 K; whereas they are respectively 354 K and 345.8 K for the corneas that were cooled to 21 C before exposure. The additional energy required to produce a minimal lesion in the cooled corneas is therefore sufficient to raise the cornea temperature to a level slightly higher than that associated with damage *in vivo*. These results suggest that threshold damage from exposures to multiple pulses has a substantial thermal component. The higher "damage temperatures" in the experiments on cooled enucleated eyes are possibly due a slowing down of the processes leading to the observed damage endpoint as a result of the lower ambient temperature. (Recall that the damage assessment is made 1/2 hour after exposure and the enucleated eyes are maintained in BSS at 21 C during this time, whereas the *in vivo* eyes are at their normal, or perhaps slightly higher temperature during this time, because they are taped shut.)

Nevertheless, in the Introduction it was noted that light and electron micrographs of corneas exposed to a single 80 ns pulse just above the damage threshold had features consistent with both thermal and mechanical (e.g., acoustic) damage. (Farrell et al, 1989; Farrell et al, 1990) These, as well as the present experiments were conducted under conditions where the energies were too low to cause optical breakdown or tissue ablation; however, large temperature gradients at the anterior surface resulting from these short exposures could produce thermoelastic pressure transients. (Sigrist and Kneubühl, 1978; Berthelot and Busch-Vishniac, 1985; Esenaliev et al, 1993; Doukas and Flotte, 1996) The thermoelastic stress wave generated by laser absorption at a free surface is bipolar; i.e., it consists of a compression wave followed by a tensile wave. (Berthelot and Busch-Vishniac, 1985; Esenaliev et al, 1993; Doukas and Flotte, 1996) According to A. G. Doukas (private communication), tensile stress is more damaging than compressional stress. According to him, rise times and other conditions being equal, it takes about an order of magnitude less tensile stress amplitude to cause cellular damage than it does for compressional stress; however, there are no published data to support this contention. Disruptions of the superficial wing cells of the epithelium noted by Farrell et al (1989; 1990) are consistent with the type of effect that might result from the passage of a tensile stress wave. At the same time, the loss of well-defined organelles and vacuolation of the anterior epithelial cells shown in electron micrographs is consistent with thermal damage. It is known that stress waves can decrease cell viability and increase cell permeability. (Lee et al, 1996; Doukas and Flotte, 1996). Thus thermoelastic stress waves may not be the primary damage mechanism, but they may serve to potentiate thermal damage. If this is indeed the case, it may explain why the temperature rises associated with the damage threshold are lower than those that would be predicted by the modified critical temperature law that describes damage for longer exposures.

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## Conclusions

Threshold damage to the corneal epithelium resulting from exposure to sequences of 80 ns pulses of $CO_2$ laser radiation is correlated by an empirical power law of the form $ED_{th} = CN^{-\alpha}$ in which $ED_{th}$ is the threshold energy density and $N$ is the number of pulses in the sequence. Although the empirical constants $C$ and $\alpha$ appear to differ slightly for the thresholds obtained at 10 Hz and at 16 Hz, both fits fall within the ±10 percent accuracy estimated from the bracketing procedure used to determine the thresholds. If both sets of data are assumed to be part of the same population, the empirical constants are $C = 2955$ J-m$^{-2}$-pulse$^{-1}$ (295.5 mJ-cm$^{-2}$ - pulse$^{-1}$) and $\alpha = 0.178$ (R = 0.984). The values for the constant $C$ obtained either for the individual data sets or the combined data all differ by less than 5% from the measured damage threshold energy density for a single pulse. Temperature calculations reveal that the maximum temperature increase on the beam axis, 10 μm beneath the anterior tear surface, resulting from the different threshold exposures is essentially constant, which suggests that the damage mechanism has a substantial thermal component and can be described by a critical temperature damage model. Additional damage determinations for multiple-pulse exposures using cooled corneas were done with the goal of clarifying the damage mechanism. The results of these experiments bolstered the view that there is a substantial thermal component to the damage, at least for sequences of pulses.

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## References

Bargeron, C. B. ; McCally, R. L. ; Farrell, R. A. Calculated and measured endothelial temperature histories of excised rabbit corneas exposed to infrared radiation. Exp Eye Res. 32: 241-250; 1981a.

Bargeron, C. B. ; Farrell, R. A. ; Green, W. R. ; McCally, R. L. Corneal damage from exposure to IR radiation: rabbit endothelial damage thresholds. Health Phys. 40: 855-862; 1981b.

Bargeron, C. B. ; Deters, O. J. ; Farrell, R. A. ; McCally, R. L. Epithelial damage in rabbit corneas exposed to $CO_2$ laser radiation. Health Phys. 56: 85-95; 1989.

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Berthelot Y. H. ; Busch-Vishniac, I. J. Laser-induced thermoacoustic radiation. J. Acoust. Soc. Am. 78: 2074-2082; 1985.

Brownell, A. S. ; Stuck, B. E. Ocular and skin hazards from $CO_2$ laser radiation. Proceedings of the 9th Army Science Conference 1: 123-138; 1974.

Carslaw H. S. ; Jaeger, J. C. Conduction of heat in solids. Oxford: Clarendon Press; 1959.

Chang H. C. ; Dedrick, K. G. Corneal damage thresholds of $CO_2$ laser radiation. Appl. Optics 8: 126-127; 1969.

Doukas, A. G. ; Flotte, T. J. Physical characteristics and biological effects of laser-induced stress waves. Ultrasound in Med. and Biol. 22: 151-164; 1996.

Egbert, D. E. ; Maher, E. F. Corneal damage thresholds for infrared laser exposure: experimental data, model calculations, and safety standards. Brooks AFB: USAF School of Aerospace Medicine, SAM-TR-77-29; 1974.

Esenaliev, R. O. ; Oraevsky, A. A. ; Letokov, V. S. ; Karabutov, A. A. ; Malinsky, T. V. Studies of acoustical and shock waves in the pulsed laser ablation of biotissue. Lasers in Surg. Med. 13: 470-484; 1993.

Farrell, R. A. ; McCally, R. L. ; Bargeron, C. B. ; Green, W. R. Structural alterations in the cornea from exposure to infrared radiation, U.S. Army Medical Research and Development Command, Fort Detrick, MD 21701-5012 ; 1989.

Farrell, R. A. ; Bargeron, C. B. ; McCally, R. L. ; Green, W. R. Corneal effects produced by IR laser radiation. In Laser Safety, Eyesafe Laser Systems, and Laser Eye Protection, Proceedings of the SPIE. Los Angeles, California: 1207; 1990: 59-70.

Ham W. J. ; Mueller, H. A. The ocular effects of single pulses of 10.6 μm and 2.5 - 3.0 μm Q-switched laser radiation, Rept. to Los Alamos Sci. Lab L-Division; 1976.

Lee, S. ; Anderson, T. ; Zhang, H. ; Flotte, T. J. ; Doukas, A. G. Alteration of cell membrane by stress waves *in vitro*. Ultrasound in Med. and Biol. 22: 1285-1293; 1996.

Lund, D. L. ; Stuck, B. E. ; Beatrice, E. A. Biological research in support of project MILES. Presidio of San Franscisco, CA: Letterman Army Institute of Research Rept. No. 96; July 1981.

Maher, E. F. Transmission and absorption coefficients for ocular media of the rhesus monkey, USAF School of Aerospace Medicine, Brooks AFB SAM-TR-78-32; 1978.

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**Submitted to Health Physics-In Review**

Maurice, D. M. The cornea and sclera. In The Eye, H. Davson (ed.). Orlando: Academic Press; 1984.

McCally, R. L. ; Farrell, R. A. ; Bargeron C. B. Cornea epithelial damage thresholds in rabbits exposed to Tm:YAG laser radiation at 2.02 μm. Lasers in Surgery and Medicine 12: 598-603; 1992.

McCally R. L . ; Bargeron C. B. Epithelial damage thresholds for sequences of 80 ns pulses of 10.6 μm laser radiation. Jour. of Laser Appl. 10: 137-139; 1998.

Morse, P. M. ; Feshbach, H. Methods of theoretical physics. New York: McGraw-Hill; 1953.

Peabody, R. R. ; Rose, H. ; Peppers, N. A. ; Vassiliadis, A. Threshold damage from $CO_2$ lasers. Arch. Ophthalmol. 82: 105-107; 1969.

Peppers, N. A. ; Vassiliadis, A. ; Dedrick, L. G. ; Chang, L. G. ; Peabody, R. R. ; Rose, H. ; Zweng, H. C. Cornea damage thresholds for $CO_2$ laser radiation. Appl. Optics 8: 377-381; 1969.

Rosenbluth R. F. ; Fatt, I. Temperature measurements in the eye. Exp. Eye Res. 25: 325-341; 1977.

Sigrist M. W. ; Kneubühl, F. K. Laser generated stress waves in solids. J. Acoust. Soc. Am. 64: 1652-1663; 1978.

Zuclich, J. A. ; Blankenstein, M.F. ; Thomas, S. J. ; Harrison, R. F. Corneal damage induced by pulsed $CO_2$ laser radiation. Health Phys. 47: 829-835; 1984.

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## Figure Captions

1. Lesion resulting from an exposure to 128 pulses from a $CO_2$-TEA laser at a pulse repetition frequency of 16 Hz. The energy density was 1440 J-m$^{-2}$ (144 mJ-cm$^{-2}$), which is 30% greater than the damage threshold.

2. The dependence of the threshold energy density per pulse on the number of pulses at pulse frequencies of 10 and 16 Hz. The lines are least-squares fits to a power law of the form $ED_{th} = CN^{-\alpha}$. The corresponding values of $C$ and $\alpha$ are given in the text. The error bars are ±10 percent of the experimental threshold values (■ – 10 Hz, ▲ – 16 Hz) and represent the estimated accuracy of the bracketing procedure used to determine the thresholds.

3 Calculated temperature histories on the beam axis 10 μm beneath the tear surface at the damage threshold exposure for sequences of 32 pulses, and (b) 128 pulses at 16 Hz.

4 The calculated maximum temperature rises on the beam axis, 10 μm beneath the tear surface for the damage threshold exposure. The lines show the mean values of $\Delta T_{max}$ for the two exposure conditions (■ – 10 Hz, ▲ – 16 Hz).

**Appendix B**
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Table 1: Threshold energy densities and calculated maximum temperature rises for sequences of 80 ns pulses.

| Number of Pulses | Pulse Repetition Frequency (Hz) | $ED_{th}$ ($mJ\text{-}cm^{-2}\text{-}pulse^{-1}$) | $d_{1/e}$ (mm) | $\Delta T_{max}$ (C)[a] |
|---|---|---|---|---|
| 1 | – | 307 | 3.72 | 30.25 |
| 2 | 10 | 235 | 3.48 | 25.68 |
| 8 | 10 | 228 | 3.80 | 32.00 |
| 32 | 10 | 154 | 3.78 | 29.15 |
| 128 | 10 | 136 | 3.41 | 32.45 |
| 1024 | 10 | 95 | 3.21 | 26.60 |
| 2 | 16 | 265 | 3.62 | 29.73 |
| 8 | 16 | 205 | 3.75 | 31.26 |
| 32 | 16 | 150 | 3.73 | 32.90 |
| 128 | 16 | 105 | 3.82 | 32.80 |
| 1024 | 16 | 85 | 3.58 | 35.06 |

[a] Calculated on the beam axis, 10 μm beneath the anterior tear surface.

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Table 2: Threshold Energy Densities and Calculated Maximum Temperature Rises for Enucleated Eyes at 21 C.

| Number of Pulses | Pulse Repetition Frequency (Hz) | $ED_{th}$ ($mJ\text{-}cm^{-2}\text{-}pulse^{-1}$) | $d_{1/e}$ (mm) | $\Delta T_{max}$ (C) [a] |
|---|---|---|---|---|
| 8 | 16 | 393 | 3.58 | 59.8 |
| 32 | 16 | 236 | 3.58 | 51.6 |

[a] Calculated on the beam axis, 10 μm beneath the anterior tear surface.

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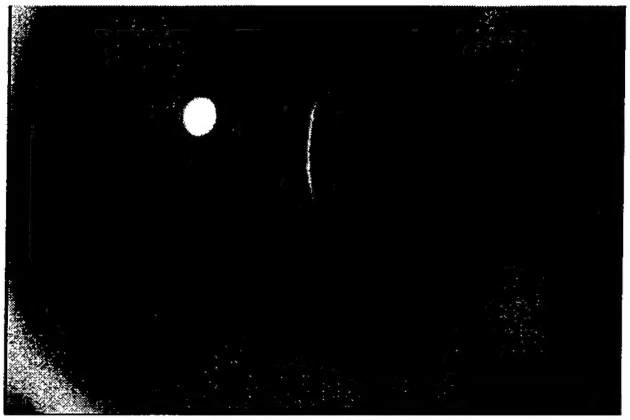

Figure 1

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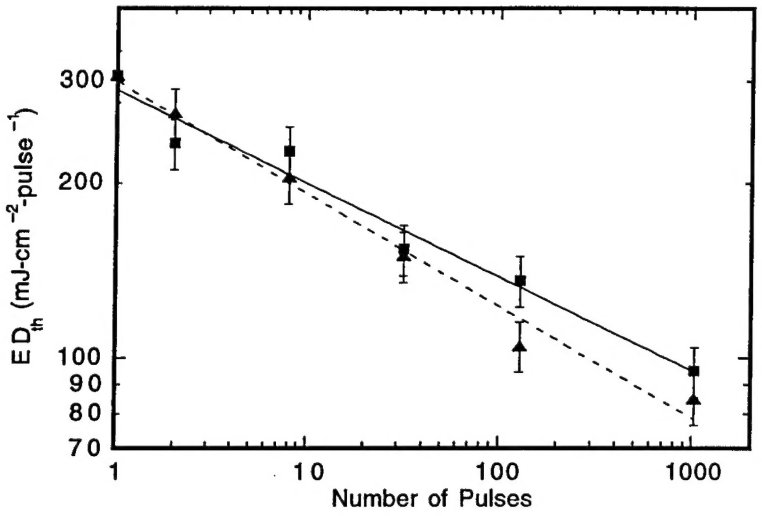


Figure 2

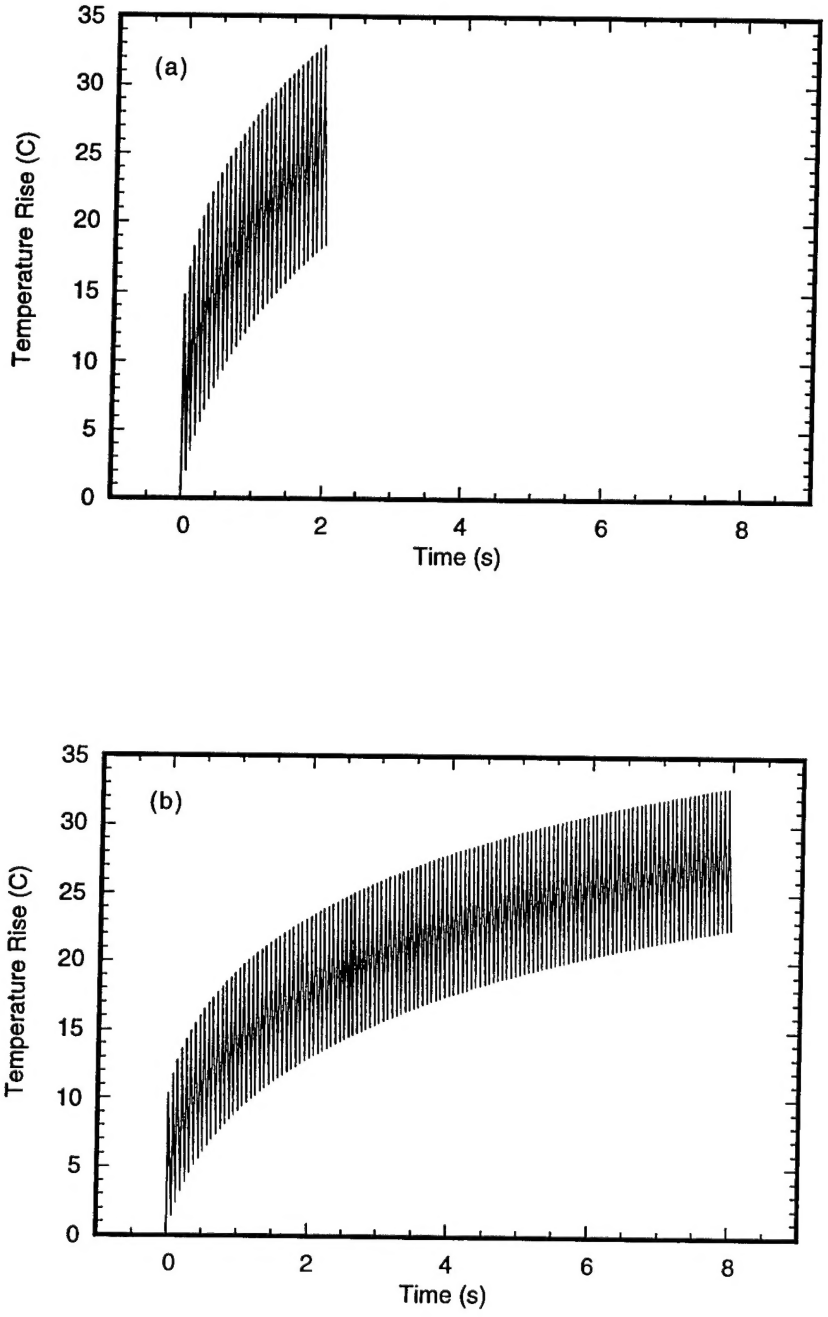
(a)
Temperature Rise (C)
Time (s)
0
5
10
15
20
25
30
35
0
2
4
6
8
(b)
Temperature Rise (C)
Time (s)
0
5
10
15
20
25
30
35
0
2
4
6
8

Figure 3

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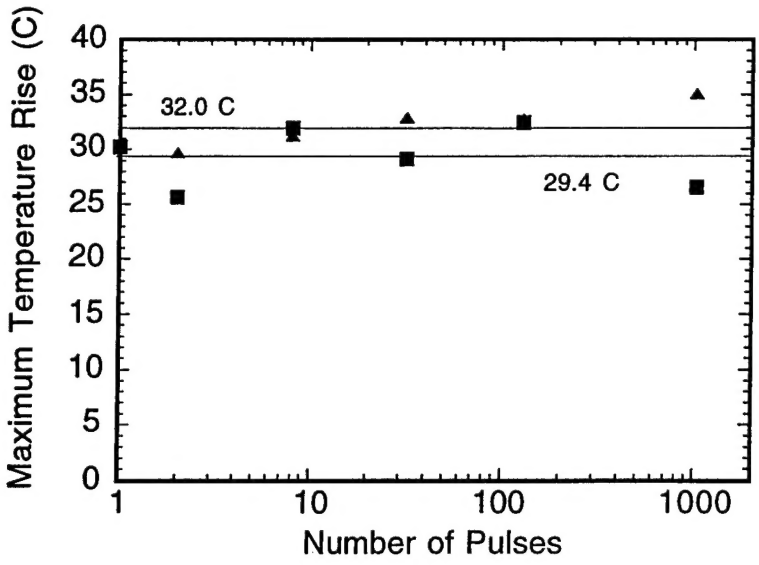


Figure 4